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# STUDIES ON THE INTRAVASCULAR THROMBOPLASTIC EFFECT OF TISSUE SUSPENSIONS IN MICE

## I. THE REACTION OF MICE TO INTRAVENOUS INJECTIONS OF A SEDIMENTABLE TISSUE COMPONENT

LEWIS THOMAS.

*From the Department of Pediatrics, Johns Hopkins University Medical School, and  
The Harriet Lane Home for Invalid Children, Baltimore, Maryland*

The property of blood to maintain its normal fluid state as it circulates through the vascular compartment is generally assumed to be the result of an equilibrium system, in which opposing forces, those which initiate and those which inhibit coagulation, are kept constantly in balance. The nature of the factors which participate in the maintenance of this equilibrium, and the mechanisms which may displace the balance to one direction or the other, are still subjects for speculation. The available methods for studying the physical state of the blood *in vitro* are limited not only by the complexity of the coagulation mechanism itself, but also by the probable absence of unknown factors whose participation may be crucial. In order to investigate the forces involved in the equilibrium, it is desirable to have methods which deal with the living animal and which permit the observation of experimentally induced deviations from the normal, *in vivo*.

It has long been known that the intravenous injection of tissue extracts in animals may cause severe toxic reactions, which are associated with the widespread formation of intravascular thrombi (1, 2), presumably due to the presence of thromboplastic substances in the tissue extracts.

During the course of an unrelated study of neurotropic viruses in this laboratory, it was observed that the intravenous injection of dilute suspensions of mouse brain tissue produced, with great regularity, a series of characteristic and easily recognizable symptoms in mice. For a period of approximately 20 seconds after injection, the animals appeared to be entirely well. They then suddenly exhibited grossly incoordinated movements, followed within a few seconds by generalized clonic convulsions and coma. The onset of these symptoms was associated with an abrupt change in the appearance of



circulating blood in the smaller vessels of the ear and mesentery, as seen by direct microscopic examination. The blood slowed sharply, and, in many vessels, came to a complete standstill, with the formation of irregular masses of apparently aggregated blood cells. At the same time, the coagulation time of the blood was found to be markedly shortened. These changes were, in most instances, transitory; within a period of some seconds the blood began to move through the vessels again, and soon became normal in appearance in vessels which had apparently been occluded a few seconds earlier. The clotting time, several minutes later, was found to be prolonged considerably beyond normal.

It was then found that when mice were given a preliminary injection of small amounts of brain tissue suspension, insufficient to cause symptoms, they became resistant within a few minutes to the toxic effect of much larger doses of the same material. This state of resistance was associated with a prolongation of coagulation time similar to that which occurred following the injection of larger, convulsion-producing doses.

The neurological symptoms in mice, and the observed changes in the appearance of circulating blood in the peripheral vessels, were completely prevented by the administration of heparin.

It is the purpose of the present paper to describe these events in detail, and to present certain related observations which bear upon the physiological disturbances involved.

#### METHODS

Two types of tissue suspensions were used for the injection of mice in the experiments to be reported. These were: a) crude suspensions, which consisted of ordinary saline extracts of ground tissue, centrifuged at low speed to remove gross tissue particles, and b) saline suspensions of the tissue component which was sedimentable by centrifugation of crude suspensions at 12,000 R.P.M.

A variety of tissues was employed in the experiments, including brain, lung, heart, liver, spleen, kidney and muscle, from mouse, rabbit and human sources. Fresh tissue, or specimens which had previously been stored in the frozen state at  $-60^{\circ}\text{C}.$ , were weighed and then ground in a mortar with an abrasive. Physiological saline solution was added to the ground tissue in a volume sufficient to

produce a 10 per cent suspension. The suspension was centrifuged at 2,000 R.P.M. for 10 minutes. The supernatant fluid will be referred to in this paper as the "crude suspension".

The crude suspension was centrifuged at 12,000 R.P.M. for 30 minutes on a motor-driven angle centrifuge, in an environment of 4°C. The supernatant fluid was discarded, and the pellet of sediment was resuspended in saline solution equal to the original volume. This suspension will be referred to as "the sedimentable tissue component".

For most of the experiments to be reported, tissue suspensions of either type were prepared in quantities of 100 ml. or more, distributed in 8 ml. amounts in lusteroid tubes, and stored at -60°C. Tissue suspensions stored in this manner showed little or no deviation from their original activity when injected into mice, after storage for 4 weeks.

Mice were injected intravenously, in the tail vein, with 0.2 ml. of the material under test. This amount was administered within two seconds. The animals were then observed carefully for a period of at least two minutes. It was found that when symptoms occurred at all, they appeared in almost every instance during the first minute after injection. Reactions were regarded as positive when mice exhibited gross ataxia, falling, and convulsive movements of the extremities. At least two mice were used for each material tested, and when endpoints were being determined in titrations of tissue suspensions four or five mice were injected.

Some variation in susceptibility to the effect of tissue suspensions was encountered when mice of different sizes were tested. Large mice were sometimes unaffected by doses which produced ataxia and convulsions in smaller mice. For this reason, mice weighing between 20 and 25 grams were used for all of the experiments to be reported. The mice were white swiss stock, obtained from a single breeder.

In the text and tables to follow, the dilutions of tissue suspension will be referred to on the basis of the original weight of whole tissue. For example, the 10 per cent suspension will be designated as the 1-10 dilution, and two-fold dilutions of this as 1-20, etc.

#### DESCRIPTION OF THE REACTION IN MICE

The following description of symptoms applies not only to mice injected with brain tissue suspensions, but also to animals receiving

suspensions of the sedimentable component of a variety of other tissues, as will be shown in a subsequent section.

For a period of between 15 seconds and one minute following the injection, the mice appeared to be unaffected and ran about in a normal manner. At the end of this period, they suddenly stopped in their tracks and began to weave their heads from side to side. After a few seconds, they exhibited incoordinated, grossly ataxic running movements and usually fell to one side. Then, in most instances, they developed violent convulsive movements of the extremities, usually lasting for less than a minute but sometimes recurring at intervals over a period of several minutes. Following the convulsions, the animals lay quietly on their sides or backs, completely immobile except for respirations. Although they appeared to be deeply comatose at this time, many of the mice would undergo a new series of convulsive movements if painful stimuli were applied to the feet or tail. They remained motionless for several minutes, and some of the animals, especially those which received concentrated doses of tissue suspension, died at this time. The majority, however, after about five minutes began to make slow clumsy movements, and after ten minutes were usually on their feet again. At this time, it was not uncommon to see animals with complete paralysis of both hind legs, or with constant involuntary turning or rolling movements. Gradually, after about fifteen more minutes, these symptoms disappeared, and at the end of an hour the mice seemed to be entirely normal.

Variations from the above typical reaction were occasionally observed. When large doses of tissue suspension were injected, the interval preceding the onset of symptoms was shortened and some of the mice underwent a brief, severe convulsion and died within a few seconds, without exhibiting other neurological symptoms. On the other hand, when the suspensions were diluted almost to the limit of their toxic effect, the interval preceding the symptoms was prolonged and the animals showed milder symptoms of ataxia and weakness, with a few brief convulsive movements of the extremities. These mice often recovered within a few seconds.

The constancy with which the reaction could be elicited in large groups of mice, with individual samples of tissue suspension, is illustrated by the following experiment. Two-fold serial dilutions of

two separate suspensions of mouse brain tissue, extending from 1-10 to 1-640, were prepared in physiological saline. Groups of 12 mice were injected intravenously with 0.2 ml. of each dilution, and the reactions were recorded. The results are shown in Table I. It will be seen that with only two exceptions all of the mice in each group reacted similarly to the injected suspensions. The exceptions occurred in the groups which received dilutions corresponding to the endpoint in each titration. The first suspension had its endpoint at the dilution of 1-160, and reactions occurred in eleven of the twelve mice in this group. With the second suspension, all of the twelve mice receiving the 1-80 dilution showed reactions, while one of the group injected with the next higher dilution had a reaction. Each

TABLE I  
*Occurrence of Ataxia and Convulsions in Groups of Mice Injected with Varying Dilutions of Mouse Brain Tissue Suspensions*

TISSUE SUSPENSION	DILUTION OF SUSPENSION*						
	1-10	1-20	1-40	1-80	1-160	1-320	1-640
1	12/12†	12/12	12/12	12/12	11/12	0/12	0/12
2	12/12	12/12	12/12	12/12	1/12	0/12	0/12

\* Dilution figures are based on the original weight of tissues.

† Numerator refers to number of mice exhibiting reaction of ataxia and convulsions. Denominator refers to number of mice tested in each group.

of the other dilutions of the suspensions produced uniform results in all mice. These findings indicated that the method of titrating suspensions by two-fold dilution yielded reactions which were sufficiently reproducible to permit comparisons to be made between the activity of different suspensions.

It was of interest to determine how closely the length of the time interval preceding symptoms could be correlated with the concentration of tissue suspension. Two-fold dilutions of a 10 per cent suspension of mouse brain tissue were prepared in physiological saline, and 0.2 ml. of each dilution were injected into mice. The time between the injection and the first symptoms was determined with a stop-watch. The results are shown in Table II. A considerable degree of correlation existed between the concentration of tissue suspension and the

length of the interval required for the appearance of symptoms. The average interval in mice receiving the 1-10 dilution was approximately 19 seconds, compared with 26 seconds following the 1-20 dilution, 33 seconds with the 1-40 dilution, and 58 seconds with the 1-80 dilution.

Reactions were not produced when mice were injected by any route other than intravenous. Large doses of concentrated tissue suspension caused no symptoms when administered subcutaneously and intraperitoneally. Intracerebral injection produced, in some mice, immediate neurological symptoms accountable to local injury of the

TABLE II  
*Effect of Dosage of Mouse Brain Tissue Suspensions on the Time Interval Preceding the Reaction in Mice*

DILUTION OF SUSPENSION	NUMBER OF MICE	TIME INTERVALS IN INDIVIDUAL MICE	AVERAGE TIME INTERVAL
1-10*	6	20", 20", 20", 19", 18", 20"†	19.5"
1-20	5	26", 27", 25", 27", 28"	26.6"
1-40	7	35", 30", 30", 45", 40", 35", 20"	33.5"
1-80	5	55", 60", 60", 70", 45"	58"

\* Figures indicating dilution are based on the original weight of brain tissue.

†Numbers refer to the interval between the time of injection and the appearance of symptoms, expressed in seconds.

brain, but the symptoms did not resemble the reaction following intravenous inoculation.

### *The Effect of Anticoagulant Drugs*

It seemed probable that the symptoms in the injected mice were due to the thromboplastic action of tissue suspensions within the circulating blood, and it was therefore of interest to study the effect on the reaction of two anti-coagulant drugs, heparin and congo red.

A commercially prepared solution of heparin, containing 1.0 mg. of heparin in 10 ml., was diluted serially in physiological saline, and portions of each dilution were mixed with equal volumes of a 10 per cent suspension of mouse brain tissue. Each mixture was then tested by the intravenous injection of 0.2 ml. into four mice. The results are indicated in Table III. It was found that heparin, in amounts as

small as 0.004 mg. per mouse, afforded complete protection against the toxic effect of mouse brain tissue suspension.

The protective action of heparin could also be demonstrated when small amounts of this drug were injected intravenously, several minutes before the injection of tissue suspensions. Moreover, the effect was demonstrable when heparin was injected 15 to 20 seconds after the suspensions, provided that it was given before the onset of symptoms. When the injection of heparin was delayed until after the appearance

TABLE III  
*Protective Action of Intravenous Heparin and Congo Red Against Toxic Effect of Mouse Brain Tissue Suspensions in Mice*

DRUG	DOSE	REACTION*
	mg.	
Heparin	0.25†	0
	0.025	0
	0.004	0
	0.002	+
	0	+
Congo Red	5.0‡	0
	1.0	+
	0	+

\* In this and the following tables the reactions of injected mice are expressed by the symbols: + = ataxia and convulsions following injection, 0 = no symptoms.

† Indicates amount of heparin contained in 0.2 ml. of tissue suspension injected.

‡ Indicates amount of congo red injected 5 minutes before tissue suspension.

of ataxia and convulsions it was entirely without effect, and animals which had begun to exhibit symptoms showed no change in their subsequent course.

It has been reported that congo red, when given in large doses, renders the blood of animals incoagulable, although smaller doses may actually increase the coagulability of the blood (3). The mechanism underlying these effects is not known. Similar changes in coagulability were observed in this laboratory, following the intravenous injection of varying quantities of congo red. The injection of 1.0 mg., in a 1 per cent solution, resulted in reductions of the clotting time in mice to one minute or less as compared with the normal clotting time

of 3 to 5 minutes. In contrast, 5.0 mg. of the same material caused a prolongation of clotting time to 2 hours or longer.

Four mice were given intravenous injections of 1.0 mg. of congo red, in a 1 per cent solution, and four other mice received 5.0 mg. Five minutes later, each animal was tested by the intravenous injection of brain tissue suspension, in a dilution of 1-20, and at the same time four untreated mice were similarly tested. The results are shown in Table III. It will be seen that each of the animals receiving 1.0 mg. of congo red exhibited ataxia and convulsions following the injection of tissue suspension. In contrast, the mice which were given 5.0 mg. of congo red showed no symptoms. It was thus evident that doses of this drug which were sufficient to produce an anticoagulant effect also provided protection against the toxic action of tissue suspensions.

#### *The Appearance of Circulating Blood During the Reaction, in Mice*

Two methods were employed for the microscopic examination of circulating blood within the smaller blood vessels, in living mice. One group of animals was anaesthetized with nembutal, and a portion of the intact mesentery was arranged on a stage under the high-dry objective of a microscope, using the method described by Youngner and Nungester (4). In a second group, the mice were immobilized without anaesthesia upon a glass plate with scotch tape, and one ear was confined beneath the objective. In both groups, the blood vessels within a single area were observed constantly for at least one minute, and then 0.2 ml. of varying dilutions of brain tissue suspension were injected into the tail vein. Observation of the vessels was continued during the injection, and for variable periods of time thereafter.

The changes which occurred were similar in the vessels of the ear and mesentery, and were produced to an equal degree by either crude suspensions or suspensions of the sedimentable component of brain tissue.

For a period of about 20 seconds following injection, no alteration from the normal appearance of the blood within the vessels was observed. Then, at about the time when the animal, if unconfined, would be exhibiting its first symptoms, a very abrupt change occurred. The flow of blood suddenly slowed in all the vessels under observation, and ill-defined masses of apparently aggregated blood corpuscles were

seen moving sluggishly along. In some vessels the blood ceased flowing altogether, and in others it began to move back and forth in rhythmic jerking movements for a few seconds. Spasm of the vessels was not observed. The appearance of the blood at this stage resembled somewhat the so-called "sludge" phenomenon described by Knisely and his co-workers (5), in the vessels of animals under conditions of infection or trauma. Further mention of this resemblance will be made in a later section of this paper.

For about ten or fifteen seconds the blood remained in this state, either moving very slowly or not at all. After a few seconds more, the flow began again in all of the vessels under observation, at first in rhythmic jerks and then in a more even flow. Within several more seconds, the vessels and their contents appeared to be entirely normal.

When higher dilutions of the brain tissue suspension were injected, the interval preceding the change in the appearance of the blood was prolonged, and the duration of the change was briefer, but the appearance was, in general, the same as described above.

When more concentrated suspensions were injected, the flow of blood stopped completely in all vessels and death occurred in some animals within a few seconds. In two mice, which survived following the injection of a concentrated suspension, a definite thrombus was seen within one of the larger vessels, apparently a vein, after the flow of blood in other vessels had been re-established. In each instance, the thrombus appeared to be attached by a narrow base to one side of the vessel, and extended along the vessel for a short distance, filling part of the lumen. The flow of blood around the thrombus appeared to be unimpeded, and during a period of several minutes no change in the size or extent of the thrombus was observed.

Since it was shown in earlier experiments that heparin protected mice against the symptoms of ataxia and convulsions following injections of tissue suspension, it was of interest to determine whether this drug was capable of influencing the intravascular "sludging" and stasis described above. Five mice were prepared for direct visualization of mesenteric vessels, and were then injected with heparin in an amount sufficient to prevent symptoms, followed by a fully toxic dose of brain tissue suspension. No change in the normal appearance of circulating blood occurred in these animals.



Specimens of fixed tissues were obtained from mice at varying intervals after the injection of tissue suspensions, and examined for the presence of thrombi. In a few animals which received small doses of suspension and underwent a brief period of ataxia and convulsions, occasional small thrombi were encountered in the capillaries of the brain. In other animals similarly treated, no thrombi could be found in any of the tissues examined, including brain, kidney, heart, lung, liver and spleen. In animals which were injected with concentrated doses of suspension and died within a few minutes after injection, numerous thrombi were seen in the small vessels of many tissues.

### *Sedimentability of the Active Tissue Component*

The component in crude suspensions of mouse brain tissue which produced the reaction under study was composed of particles which could be sedimented by centrifugation at high speed.

This finding is illustrated by the following experiment. A 10 per cent suspension of mouse brain tissue was centrifuged at 2,000 R.P.M. in a horizontal centrifuge for 10 minutes. The supernatant fluid was then divided into two portions. One portion was centrifuged at 12,000 R.P.M. for 30 minutes. The supernatant fluid was removed, and the packed sediment was resuspended in physiological saline to the original volume and recentrifuged at low speed to remove gross particles. Serial two-fold dilutions in saline were made with the original crude suspension, the supernatant fluid following high-speed centrifugation, and the resuspended sediment from the latter. Each of these dilutions was tested by intravenous injection in mice. The results are shown in Table IV.

It will be seen that the crude suspension produced typical reactions in a dilution of 1-160. The supernatant fluid after centrifugation of this suspension at 12,000 R.P.M. failed to produce reactions in any dilution. The reconstituted sediment possessed all of the activity which was demonstrable in the original suspension, and produced, like the latter, reactions in the dilution of 1-160.

The foregoing observations do not provide any information as to the relative size of the tissue particles which were contained in the suspensions of the sedimentable tissue component. It is obvious that centrifugation of crude tissue suspensions at 12,000 R.P.M. must bring down

particles of many different sizes. The sedimentability of the active tissue component at various speeds below 12,000 R.P.M. was found to vary in crude suspensions of different tissues. In some preparations a considerable proportion of the component could be sedimented at 8,000 R.P.M., and in a few suspensions even 5,000 R.P.M. brought down much of the component.

### *Occurrence of the Active Component in Other Tissues*

Suspensions of the sedimentable component in a variety of other tissues, from mice, rabbits and human beings, were prepared by centrifugation of crude suspensions at 12,000 R.P.M. and resuspension of the

TABLE IV  
*Sedimentability of the Tissue Component Causing Ataxia and Convulsions in Mice, in a Suspension of Mouse Brain Tissue*

COMPONENT OF TISSUE SUSPENSION	DILUTION OF SUSPENSION					
	1-10	1-20	1-40	1-80	1-160	1-320
Supernate: 2,000 R.P.M.....	+	+	+	+	+	0
Supernate: 12,000 R.P.M.....	0	0	0	0	0	0
Sediment: 12,000 R.P.M.....	+	+	+	+	+	0

\* Symbols refer to the presence or absence of reactions in mice injected with the indicated dilution of tissue suspension.

sediment in saline. Serial dilutions of each suspension were then tested for the production of ataxia and convulsions in mice. The results are shown in Table V. It will be seen that the reaction was produced not only by brain but also by mouse lung, kidney, and spleen tissue. Moreover, identical reactions were produced by suspensions of the sedimentable component of rabbit brain, lung, kidney and liver tissue, and by human brain, lung, heart, kidney and spleen.

Some variation was encountered in the titers obtained with the different preparations. For example, brain and lung tissue appeared to possess more of the active tissue component than the other organs tested in each species. In contrast, the reaction was produced only by the lowest dilution of rabbit liver, and did not occur with mouse liver. The component was not demonstrable in rabbit striated muscle.

*The Effect of Heat on the Activity of Tissue Suspensions*

The effect of exposure to various temperatures on the activity of tissue suspensions from various sources was tested. Ten per cent suspensions of mouse brain, rabbit brain, human brain and human lung tissue were heated for 30 minutes at 56°C., 60°C., and 80°C. In each instance, both crude suspensions and suspensions of the sedimentable tissue component were tested simultaneously. After

TABLE V

*Production of Ataxia and Convulsions in Mice by Suspensions of the Sedimentable Tissue Component of Various Species and Organs*

TISSUE SUSPENSION		DILUTION OF TISSUE SUSPENSION					
Species	Organ	1-10	1-20	1-40	1-80	1-160	1-320
Mouse	Brain	+	+	+	+	+	0
	Lung	+	+	+	+	+	0
	Kidney	+	+	+	0	0	0
	Spleen	+	+	+	+	0	0
	Liver	0	0	0	0	0	0
Rabbit	Brain	+	+	+	+	+	0
	Lung	+	+	+	+	+	0
	Kidney	+	+	+	0	0	0
	Liver	+	0	0	0	0	0
	Striated muscle	0	0	0	0	0	0
Human	Brain	+	+	+	+	+	0
	Lung	+	+	+	+	+	0
	Heart	+	+	+	0	0	0
	Kidney	+	+	0	0	0	0
	Spleen	+	+	+	0	0	0

heating, two-fold serial dilutions of each suspension were made in physiological saline, and the titer determined by injection into mice.

The results, which differed considerably in different types of tissue suspension, are shown in Table VI. It will be seen that the crude suspension of mouse brain, which had an endpoint in the 1-80 dilution before heating, was not affected by 56°C., but was completely inactivated by heating at 60° and 80°C. In contrast, a suspension of the sedimentable component of this tissue was only slightly reduced in activity by exposure to 80°C.

TABLE VI  
The Effect of Heating Various Tissue Suspensions on Their Toxicity for Mice

TISSUE	SUSPENSION	TEMPERATURE: °C.	DILUTION OF SUSPENSION					
			1-10	1-20	1-40	1-80	1-160	1-320
Mouse Brain	Crude*	Unheated	+	+	+	+	0	
		56	+	+	+	+	0	
		60	0	0	0	0		
		80	0	0				
	S.T.C.†	Unheated	+	+	+	+	0	
		56	+	+	+	+	0	
		60	+	+	+	0	0	
		80	+	+	+	0	0	
	Rabbit brain	Unheated	+	+	+	+	0	
		56	+	+	+	+	0	
		60	0	0	0	0		
		80	0	0				
	S.T.C.	Unheated	+	+	+	+	0	
		56	+	+	+	+	0	
		60	+	0	0			
		80	+	0	0			
Human brain	Crude	Unheated	+	+	+	+	+	0
		56	+	+	+	+	+	0
		60	+	+	+	0	0	
		80	+	+	+	0	0	
	S.T.C.	Unheated	+	+	+	+	+	0
		56	+	+	+	+	+	0
		60	+	+	+	+	+	0
		80	+	+	+	+	+	0
Human lung	Crude	Unheated	+	+	+	+	0	
		56	+	+	+	+	0	
		60	+	+	+	+	0	
		80	+	+	+	+	0	
	S.T.C.	Unheated	+	+	+	+	0	
		56	+	+	+	+	0	
		60	+	+	+	+	0	
		80	+	+	+	+	0	

\* Crude = Supernatant fluid after centrifugation at 2,000 R.P.M.

† S.T.C. = Sedimentable tissue component, after 12,000 R.P.M., resuspended in NaCl.

‡ Tissue suspensions heated for 30 minutes at the temperature indicated.

Similar results were obtained with the rabbit brain tissue suspensions. The crude suspension was not affected by 56°C., but was inactivated at higher temperatures. The suspension of the sedimentable component was partially inactivated at 60° and 80°C., but still retained activity in the 1-10 dilution.

Human brain tissue suspensions showed greater stability to heat than mouse or rabbit brain. The crude suspension, which had a titer of 1-160 before heating, was reduced in activity by 60° and 80°C., but still caused reactions in the dilution of 1-40. On the other hand, the

TABLE VII

*The Effect of Preliminary Injections of Small Amounts of Tissue Suspension upon the Susceptibility of Mice to Subsequent Injection of Toxic Doses*

PRELIMINARY INJECTION	SECOND INJECTION	ATAXIA AND CONVULSIONS	
		Prepared group*	Controls
Mouse brain—dil. 1-160	Mouse brain—dil. 1-40	1/12†	12/12
Mouse brain—dil. 1-160 followed by 1-40	Mouse brain—dil. 1-10	0/11	12/12
Mouse kidney—dil. 1-80	Mouse brain—dil. 1-20	0/8	8/8
Rabbit brain—dil. 1-160	Mouse brain—dil. 1-40	0/8	8/8
Human lung—dil. 1-320	Mouse brain—dil. 1-40	1/8	8/8
Mouse brain—dil. 1-160	Human lung—dil. 1-40	0/8	8/8

\* Mice which received preliminary injections of indicated tissue suspension.

† Numerator refers to number of mice showing ataxia and convulsions following the second injection. Denominator refers to number of mice tested.

suspension of the sedimentable component was not affected by heating at these temperatures.

Human lung tissue differed from the three varieties of brain tissue, in that both the crude and sedimentable component suspensions were completely resistant to heating at 80°C.

### *Induced Resistance to the Toxic Effect of Tissue Suspensions in Mice*

The smallest amount of the sedimentable component of mouse brain tissue which produced ataxia and convulsions in mice was, in most preparations, represented by dilutions of 1-80 or 1-160. It was observed that when mice were injected with dilutions which were less than the amount required to produce symptoms, they rapidly became resistant to the toxic action of more concentrated suspensions.

An illustration of this phenomenon is presented in Table VII. Twelve mice were injected with a 1-160 dilution of mouse brain tissue,

with no reaction. Five minutes later, each mouse was reinjected with the same material in a dilution of 1-40, and at the same time 12 control mice were given a similar dose. Only one of the 12 animals which had received the preliminary dilution of 1-160 showed symptoms following the second injection, while all of the control mice exhibited typical ataxia and convulsions.

The 11 mice which survived the second injection without effect were reinjected five minutes later with a dilution of 1-10, and at the same time 12 control mice were similarly injected. None of the previously injected mice showed the usual reaction of ataxia and convulsions, although some of the animals appeared somewhat weak and ruffled. In contrast, all of the control animals exhibited typical, severe reactions which in several instances were rapidly fatal.

A similar state of resistance was produced when preparatory injections of tissue from one organ were followed by toxic doses of tissue suspension derived from another organ. For example, as is shown in Table VII, mouse kidney tissue in less than toxic doses provided 8 mice with resistance to the toxic action of mouse brain tissue suspension. Moreover, it will be seen that the tissues of different species produced protection against each other, as, for example, rabbit brain against mouse brain, human lung against mouse brain, and mouse brain against human lung.

The state of resistance was not produced if the preliminary injections of tissue suspension were made by any route other than intravenous. The injection of 1.0 ml. intraperitoneally and 1.0 ml. subcutaneously, of concentrated brain tissue suspension, did not cause any increase in the resistance of mice to subsequent intravenous injections of the same tissue.

Resistance did not occur if suspensions of brain tissue which had been inactivated by heating were used for the preliminary injection. It was necessary to employ active suspensions which were only slightly less concentrated than the dose required to produce obvious symptoms. For example, when a given suspension of brain tissue possessed a titer of 1-80, resistance could be induced by injecting the 1-160 dilution and sometimes by the 1-320 dilution, but no effect was demonstrable following the 1-640 dilution.

The state of resistance was first demonstrable within two or three minutes after the preliminary injection, and was present with greatest

regularity at a period between five and ten minutes following injection. After 30 minutes it was demonstrable in a smaller proportion of animals, and at the end of one hour it could no longer be demonstrated.

During the period of resistance, the injection of concentrated tissue suspensions produced little or no change in the appearance of the circulating blood in the vessels of the ear and mesentery, in contrast to the stasis and "sludging" which was observed in unprepared mice.

TABLE VIII

*Effect of the Intravenous Injection of Mouse Brain Tissue Suspension on Clotting Time in Mice*

DILUTION OF SUS- PENSION	TIME AFTER INJECTION	CLOTTING TIME (INDIVIDUAL MICE)					
1-20	15 seconds	< 5 sec.	< 5 sec.	< 5 sec.	< 5 sec.	< 5 sec.	< 5 sec.
	5 minutes	> 2 hrs.	> 2 hrs.	> 2 hrs.	> 2 hrs.	> 2 hrs.	> 2 hrs.
	15 minutes	11 min.	8 min.	5 min.	14 min.	12 min.	10 min.
1-160	15 seconds	10 sec.	20 sec.	24 sec.	30 sec.	40 sec.	30 sec.
	5 minutes	> 2 hrs.	> 2 hrs.	> 2 hrs.	10 min.	12 min.	8 min.
	15 minutes	11 min.	8 min.	5 min.	3 min.	5 min.	3 min.
Normal mice.....		4 min.	5 min.	3 min.	4 min.	3 min.	3 min.

*The Effect of Injections of Brain Tissue Suspension on the Coagulability of the Blood in Mice*

In order to obtain an explanation for the phenomenon of induced resistance described in the preceding section, a study of the coagulation time of the blood of mice following injection of tissue suspensions was undertaken.

Groups of mice were injected intravenously with various dilutions of a suspension of the sedimentable component of mouse brain tissue. Clotting time determinations were made in sample mice at intervals of 15 seconds, 5 minutes, and 15 minutes after the injection, by placing 0.5 ml. of heart's blood in a small glass test tube, and tilting the tube at intervals until complete clotting was apparent. By this method the clotting time in a group of twelve normal mice was found to range between 3 and 8 minutes.

The results which were obtained in two illustrative experiments are shown in Table VIII. In the first experiment, 18 mice were injected

with 0.2 ml. of a 1-20 dilution of tissue suspension. Each of these animals developed a severe convulsive reaction, which started between 15 and 20 seconds after the injection. At this time, the clotting time in six animals was so rapid that the blood began to clot within the syringe while being drawn. The clotting time was assumed to be less than 5 seconds. Five minutes later, the clotting time in six other mice was determined. In each instance, the blood failed to clot within a period of 2 hours. Fifteen minutes after the injection, the clotting time of the remaining six mice ranged between 5 and 14 minutes.

Similar effects were produced by a smaller dose of the tissue suspension. Eighteen mice were injected with the 1-160 dilution, which caused no symptoms. Fifteen seconds later, the clotting time in six mice was much accelerated, varying between 10 and 40 seconds. At five minutes, three of the animals had clotting times longer than 2 hours, while three others were moderately prolonged. At fifteen minutes, the clotting time in the remaining six mice was normal or nearly normal.

Additional experiments of the same nature were carried out in other groups of mice with the same general results, i.e., a sharp reduction in clotting time within the first 30 seconds, followed by a marked prolongation at five minutes, and approaching normal again after fifteen minutes. These findings offer a possible explanation for the state of resistance to toxic doses of tissue suspension which followed the injection of smaller doses. It is of interest that the maximum degree of incoagulability was observed at five minutes following injection, which was also the time at which resistance to a second injection was most regularly demonstrable.

No inhibitor of coagulation could be detected in the whole blood or serum of mice at the time when the clotting time was most prolonged. When blood from these animals was mixed with fresh blood from normal mice, the clotting time of the mixture was not significantly different from that of normal blood.

Prothrombin time determinations by the method of Quick (6) were undertaken with plasma from six mice which had been injected five minutes earlier with a 1-160 dilution of tissue suspension, and compared with the prothrombin time in six normal mice. The normal prothrombin time ranged between 9 and 12 seconds. In three of the



injected mice, the prothrombin time lay within this range, while in the other three the time was 15, 16 and 17 seconds respectively. The latter figures, although somewhat prolonged, did not seem to be sufficiently abnormal to account for the marked delay in coagulation of whole blood observed in injected mice.

*The Effect of Injections of Rabbit Brain Tissue Suspension on the Coagulability of the Blood in Rabbits*

Normal rabbits, weighing approximately 6 pounds, were injected intravenously with various dilutions of suspensions of the sedimentable component of rabbit brain tissue, in doses of 5 or 10 ml. per rabbit. Considerable variation was encountered in the susceptibility of individual rabbits to the toxic action of tissue suspensions, as compared with the uniform responses which had been observed in mice. Further variation could be produced by varying the speed with which the suspensions were injected; rapidly introduced suspensions caused symptoms more frequently than slow injections. When symptoms occurred, they consisted of severe generalized convulsions which usually began within 30 and 60 seconds after the injection and lasted intermittently for about 1 minute. In almost every instance, the convulsions were followed by immediate death, and were associated with widespread intravascular clotting.

Blood was obtained from the heart several minutes before the injection of tissue suspension, and at varying intervals following injection. Clotting time was determined with 0.5 ml. samples, using the method previously described. The clotting time in twenty-five normal rabbits ranged between 3 and 10 minutes.

The results obtained in a series of 25 rabbits were not uniform, due largely to the difficulty of selecting an adequate dose of tissue suspension for individual animals without causing generalized convulsions and death. However, in 12 rabbits of this series, definite changes in the coagulability of blood were observed following injection, and these changes were similar to those observed in mice. Within the first minute after injection the clotting time became accelerated, and five minutes later it was prolonged beyond normal. The delay in coagulation persisted for varying lengths of time during the following hour.

A detailed summary of the changes in clotting time in four rabbits is shown in Table IX. Each of these animals was injected with 10 ml. of a 1-40 dilution of rabbit brain sedimentable tissue suspension. It will be seen that the first animal had a clotting time of 8 minutes

TABLE IX

*Effect of the Intravenous Injection of Rabbit Brain Tissue Suspension on Clotting Time and Prothrombin Time in Rabbits*

RABBIT NO.	DILUTION OF SUSPENSION	BLOOD OBTAINED	CLOTTING TIME	PROTHROMBIN TIME
				sec.
1	1-40	Before injection	8 minutes	15
		After 30 seconds	3 minutes	16
		After 5 minutes	21 minutes	14
		After 15 minutes	22 minutes	14
		After 30 minutes	> 2 hours	29
		After 45 minutes	20 minutes	13
		After 60 minutes	14 minutes	13
2	1-40	Before injection	6 minutes	12
		After 30 seconds	1 minute	
		After 10 minutes	48 minutes	12
		After 30 minutes	> 2 hours	60
		After 50 minutes	4 minutes	12
3	1-40	Before injection	6 minutes	14
		After 30 seconds	30 seconds	
		After 5 minutes	23 minutes	12
		After 15 minutes	27 minutes	14
		After 30 minutes	7 minutes	14
4	1-40	Before injection	5 minutes	
		After 1 minute	1½ minutes	
		After 5 minutes	> 2 hours	
		After 20 minutes	> 2 hours	
		After 60 minutes	4 minutes	

before injection. Thirty seconds after injection the time was 3 minutes. At five and fifteen minutes after injection, the clotting time had increased to approximately 20 minutes, and at a half-hour the blood failed to clot within 2 hours. At forty-five minutes the clotting time had decreased to 20 minutes, and at the end of the hour it was close to normal. Comparable results were obtained in the other three

rabbits indicated in Table IX, although some variation was encountered in the time intervals at which maximum prolongations of clotting time occurred.

The prothrombin times in successive plasma samples from three of the rabbits are shown in Table IX. It will be seen that the prothrombin time remained within normal limits in all except two samples. In rabbit no. 1 the prothrombin time one-half hour after injection was 29 seconds, and in rabbit no. 2, at the same interval, the time was 60 seconds. In each instance, the plasma clots were loose and granular, as compared with the firm clots observed in other plasma specimens.

No anticoagulant substance could be demonstrated in the blood of rabbits following the injection of tissue suspensions. When the relatively incoagulable blood of these animals was mixed with normal rabbit blood, no delay in the clotting time of the latter was observed. Moreover, the clotting time was not shortened by the addition of varying concentrations of salmine, which is known to act as an antagonist of heparin (7). Platelet counts remained normal throughout the reaction in three rabbits tested.

#### DISCUSSION

It has long been recognized that the intravenous injection of tissue extracts in animals may result in the formation of extensive thromboses throughout the vascular compartment, causing death within a few minutes. Such an event can be produced in mice, if considerable doses of a concentrated tissue suspension are injected. Smaller doses of the same material, however, bring about a characteristic, almost stereotyped pattern of neurological symptoms, which are transient and apparently completely reversible. Between the time of injection and the appearance of symptoms, a latent interval is always observed. The duration of this interval is quite regular and constant, for a given dose of tissue suspension, and varies directly with the concentration of material injected.

At the time when symptoms appear in the mice, certain changes occur in the physical state of the circulating blood, and can be observed directly by microscopic examination of the vessels of the ear or mesentery. The blood slows, becomes "sludge-like" in appearance, and may come to a complete standstill in many vessels. Then, after a period of seconds, it moves on again in a normal fashion.

This reaction does not occur if the mice are treated beforehand with heparin, or with anticoagulant doses of congo red. It does not occur if the tissue suspension has been inactivated by heating. Moreover, if the mice have received a preliminary sub-toxic injection of the tissue suspension they become, within a few minutes, resistant to fully toxic amounts; this state of resistance is associated with a marked prolongation of the coagulation time.

The tissue component which is responsible for the reaction in mice is sedimentable by centrifugation at high speed, and is demonstrable in a wide variety of homologous and heterologous tissues. Similar tissue components are known to contain thromboplastin (8). It is reasonable to assume that the symptoms, and the transient stasis of blood flow, are both caused by the thromboplastic action of the tissue suspensions introduced into the circulating blood, and that the acquired resistance displayed by previously injected animals is the result of the temporary coagulation defect which occurs following such injections.

The predominantly neurological character of the symptoms in mice suggests an injury to the brain. The physiological basis of this injury is, however, not clear. Is it due to actual thrombosis within cerebral vessels? If so, it is somewhat difficult to understand the rapidity and completeness with which many of the mice recover, following the reaction. Also, one might expect to find more histological evidence of thrombosis than was encountered in the brain sections examined. These objections, however, are not sufficient to exclude the possibility of cerebral thrombi as the underlying basis for the reaction. It is conceivable that scattered, small thrombi, if accompanied by temporary circulatory stasis elsewhere in the body, or if attended by local vasospasm within the brain, might account for the transient symptoms observed. Moreover, such thrombi might be extremely difficult to locate in fixed brain sections.

However, it is also possible that the same sort of intravascular changes which were observed by direct visualization, in the ear and mesenteric vessels, may take place within the brain. There may be stasis and "sludging" of blood, without necessarily the formation of organic, fixed thrombi, and such a change may result in a severe enough degree of cerebral ischemia to account for the symptoms displayed by the animals. On such a basis, it would be easier to explain

the transient and reversible course of the reaction as well as the failure to detect thrombi in fixed tissue sections. The nature of this disturbance in blood flow is, at the present time, a matter for speculation. Whether it is due to an increase in viscosity, or to obstructive aggregations of the particulate elements of the blood, or to an actual change in the colloidal state of the blood representing "partial clotting", has not been determined.

The theoretical implications of such an explanation are considerable, and perhaps a certain amount of correlary speculation is warranted. It is conceivable that a similar event may take place under other circumstances, during the course of disease or as the result of trauma, and may be the basis for certain types of tissue damage. Injury to the endothelial barrier between the blood and tissues, for example, could result in the release of a similar component of tissue into the local vascular bed, producing a local, transient disturbance in blood fluidity with the temporary effect of thrombosis, but without the formation of an actual thrombus. Moreover, it is known that normal blood contains thromboplastic substances, which are demonstrable in platelets and perhaps also in erythrocytes and white cells (9). Liberation or activation of these sources of thromboplastin might also lead to similar disturbances in blood flow.

It has already been mentioned that the blood flow changes in mice seemed to resemble the "sludging" described by Knisely and his associates (5) in the small blood vessels of animals in the course of certain acute infections, notably malaria in monkeys, and also in vessels adjacent to traumatized areas in mice. The possibility of a similar underlying mechanism seems of sufficient interest to warrant mention at this time.

In this connection it is also of interest to consider the recent studies of Friedman, Lange and Weiner on experimental frostbite and gangrene in animals (10). These workers found that the freezing of living tissue was followed by changes in the physical state of blood within the vessels of the affected area, which resembled "sludging". Gangrene of the area was prevented by the administration of heparin. It is possible that the trauma of freezing may have caused the release of thromboplastic material into the local vascular bed, resulting in blood flow changes analogous to those observed in mice following the injection of tissue suspensions.

The occurrence of decreased coagulability in animals following the injection of tissue extracts has been recognized for many years. In 1886, Wooldridge (1) reported that the injection of "tissue fibrinogen" intravenously in dogs resulted in intravascular clotting when large quantities were given. The injection of smaller doses was followed by a period in which the blood was relatively incoagulable, and during which the animals were insusceptible to the toxic effect of larger doses of the tissue extract. In 1909, similar observations were made by Mellanby (2), employing thromboplastic snake venoms instead of tissue extracts. This worker explained the increase in coagulation time on the basis of a depletion in circulating fibrinogen, resulting from intravascular clotting initiated by the injected material. In the present study, employing mice and rabbits, no new explanation of the coagulation changes has been advanced. However, it would appear that the explanation offered by Mellanby cannot be applied to the results obtained here, since the prolongation of clotting time was, in most instances, associated with a normal prothrombin time and normal clot formation. In two exceptions noted, an increase in prothrombin time appeared 30 minutes after the injection of tissue suspension in rabbits, but in both instances the prothrombin time in earlier plasma specimens was normal.

Prolongations of the coagulation time are also known to occur following anaphylactic shock in animals, and after the shock which results from injections of peptone. There is evidence that in both instances the incoagulability is due to the liberation of anticoagulant substances (11, 12). The physiological events which precipitate the characteristic symptoms in these two varieties of shock are still obscure. It is of interest that some workers have reported the inhibition of anaphylactic shock by heparin (13).

The reproducible and easily recognized symptoms displayed by mice following the injection of tissue suspensions made it possible to titrate the *in vivo* thromboplastic activity of the suspensions in a more or less quantitative fashion, thus providing a method for studying some of the properties of the tissue component involved. The observed changes in thermostability following high-speed centrifugation of crude brain tissue suspensions are of interest. Crude suspensions of mouse brain tissue were inactivated by heating at 60°C., while saline suspensions of the component sedimented by 12,000 R.P.M.

were resistant to the effect of  $80^{\circ}\text{C}$ . This observation is unexplained at the present time, but it suggests that the active component may be linked, in crude suspensions, to a thermolabile factor from which it is separated by centrifugation. It has been reported by Kidd and Friedewald (14) that a similar increase in thermostability following centrifugation occurred in an antigenic component of normal tissue which reacted in complement fixation tests with normal rabbit serum. The possible relationship between this antigenic component and the thromboplastic tissue component under study will be discussed in the paper which follows (15).

#### SUMMARY

The intravenous injection of saline suspensions of normal mouse brain tissue, in mice, caused a characteristic and reproducible reaction consisting of ataxia, convulsions and coma. The reaction was accompanied by transitory changes in the appearance of the circulating blood in the vessels of the ear and mesentery, with slowing and, in some vessels, complete cessation of blood flow. These changes were usually followed, after a short interval, by the resumption of normal blood flow.

The reaction in mice was prevented by the administration of heparin, and also by congo red in anticoagulant doses.

The tissue component which caused the reaction was completely sedimentable by centrifugation at 12,000 R.P.M., and was demonstrable in a variety of mammalian tissues. Crude suspensions of brain tissue were inactivated by heating at  $60^{\circ}\text{C}$ . Suspensions of the brain tissue component after centrifugation at high speed were resistant to temperatures as high as  $80^{\circ}\text{C}$ .

When mice were injected with small amounts of the tissue component they became, within a few minutes, resistant to the effect of fully toxic doses of the same material. This state of resistance persisted for less than one hour.

Following the injection of tissue suspensions in mice and rabbits, the clotting time of the blood was at first accelerated, then became markedly prolonged, and then returned to normal within one hour after injection.

Some of the theoretical implications of these observations are discussed.

## BIBLIOGRAPHY

1. WOOLDRIDGE, L. C.: Arch. f. Anat. u. Physiol. (Phys. Abt.), 1886, p. 397.
2. MELLANBY, J.: J. Phys., 1909, 38, 442.
3. TALLIAFERRO, I., AND HAAG, H. B.: Am. J. Med. Sci., 1937, 193, 626.
4. YOUNGNER, J. S., AND NUNGESTER, W. J.: J. Inf. Dis., 1944, 74, 247.
5. KNISELY, M. H., ELIOT, T. S., AND BLOCH, E. H.: Arch. Surg., 1945, 51, 220.
6. QUICK, A.: The Hemorrhagic Diseases and the Physiology of Hemostasis. C. C. Thomas, Springfield, Ill., 1942.
7. CHARGAFF, E., AND OLSON, K. B.: J. Biol. Chem., 1937, 122, 153.
8. CHARGAFF, E.: Advances in Enzymology, Vol. V. Interscience Publishers, Inc., New York, N. Y., 1945.
9. SILBERBERG, M.: Phys. Rev., 1938, 18, 197.
10. FRIEDMAN, N. B., LANGE, K., AND WEINER, W.: Am. J. Med. Sci., 1947, 213, 61.
11. EAGLE, H., JOHNSTON, C. C., AND RAVDIN, J. S.: Bull. Johns Hopkins Hospital, 1937, 60, 428.
12. JACQUES, L. B., AND WATERS, E. T.: J. Physiol., 1941, 99, 454.
13. MACHT, D. I.: Ann. Int. Med., 1943, 18, 772.
14. KIDD, J. G., AND FRIEDEWALD, W. F.: J. Exp. Med., 1942, 76, 543.
15. THOMAS, L.: Bull. Johns Hopkins Hospital, 1947, 81, 26.



# STUDIES ON THE INTRAVASCULAR THROMBOPLASTIC EFFECT OF TISSUE SUSPENSIONS IN MICE

## II. A FACTOR IN NORMAL RABBIT SERUM WHICH INHIBITS THE THROMBOPLASTIC EFFECT OF THE SEDIMENTABLE TISSUE COMPONENT

LEWIS THOMAS.

*From the Department of Pediatrics, Johns Hopkins University Medical School and The Harriet Lane Home for Invalid Children, Baltimore, Maryland*

In a previous paper (1) evidence was presented which indicates that the characteristic reaction of ataxia, convulsions and coma, which is produced in mice by the intravenous injection of tissue suspensions, is due to the thromboplastic action of these suspensions *in vivo*. In animals receiving lethal doses of this material, generalized intravascular clotting occurs. With smaller doses, the coagulation time is decreased at the time when the symptoms which comprise the reaction occur. The reaction may be prevented by small doses of heparin, or by anti-coagulant doses of congo red. The component of tissue causing the reaction is entirely sedimentable by centrifugation at high speed.

The present paper deals with the inactivation of the sedimentable tissue component by a factor in normal rabbit serum, which is demonstrable when saline suspensions of tissue particles are incubated with serum. It will be shown that the inhibiting factor is dependent upon the presence of calcium for its effect. Certain properties of the serum factor will be described.

### MATERIALS AND METHODS

*Serum.* Normal adult rabbits, weighing approximately six pounds each, were bled from the heart in amounts of 20 ml. each. The blood was allowed to clot at room temperature, then centrifuged at 2000 R.P.M. for 10 minutes. For prolonged storage, the serum was kept in sterile lusteroid tubes at  $-70^{\circ}\text{C}$ . In some experiments, in which the serum was to be used within 3-4 days, it was stored in the refrigerator at  $4^{\circ}\text{C}$ .

*Plasma.* Rabbit plasma was obtained by adding whole blood to one-tenth of its volume of 0.1 M potassium oxalate, and centrifuging

at 2000 R.P.M. for 10 minutes. In all experiments involving plasma, fresh specimens were used on the same day as obtained.

*Tissue Suspensions.* Saline suspensions of the sedimentable tissue component of rabbit brain, rabbit lung, mouse brain, human lung, and human brain tissue were prepared in the following manner. Freshly obtained tissues, or specimens which had previously been stored at  $-70^{\circ}\text{C}.$ , were weighed, ground in a mortar with sterile alundum, and suspended in a sufficient quantity of physiological saline to produce a concentration of 10 per cent. The suspension was centrifuged at 2000 R.P.M. for 10 minutes, and then recentrifuged at 12000 R.P.M. for 30 minutes in an angle centrifuge, at  $4^{\circ}\text{C}.$  The sediment obtained by the latter centrifugation was resuspended in physiological saline to its original volume, and the resulting suspension distributed in lusteroid tubes. All suspensions were either used in experiments on the same day as prepared, or were stored in the frozen state at  $-70^{\circ}\text{C}.$

*Titration of Suspensions.* The potency of each suspension was determined by preparing serial two-fold dilutions in physiological saline solution, and injecting 0.2 ml. of each dilution intravenously into two mice. The highest dilution which produced the typical reaction of ataxia and convulsions was designated as one unit. In the text and tables which follow, all dilutions are referred to on the basis of the original weight of whole tissue.

*Inhibition Tests.* Rabbit sera were tested in the following manner for their capacity to inhibit the activity of the sedimentable tissue component. Serial two-fold dilutions of serum were prepared in physiological saline solution, or in other diluents to be described later. To each dilution of serum was added an equal volume of the tissue suspension under study. In most experiments, the concentration of the suspension was such that the final mixture of serum and tissue to be tested contained two units of the sedimentable tissue component. For example, with suspensions which produced reactions with an endpoint in the 1-160 dilution, the final concentration of tissue suspension in each mixture was 1-80.

The mixtures of serum and tissue were placed in a water-bath at  $37^{\circ}\text{C}.$  for 1 hour, after which they were tested in mice by the intravenous injection of 0.2 ml. In all tests, appropriate control tubes of serum alone and tissue alone were included. The results in each titra-

tion of serum were recorded in terms of the final dilution of serum in each tube. The reactions in mice were recorded as "plus" if the typical reaction of ataxia and convulsions occurred, and as "zero" if no reaction occurred. At least two mice were tested with each mixture, and usually three or four mice were used for the mixtures which determined the endpoint of titrations.

## EXPERIMENTAL RESULTS

### *Inhibition of Rabbit Brain Tissue Suspensions by Rabbit Sera*

Sera from 50 normal rabbits were tested for inhibition of the toxic effect of suspensions of rabbit brain tissue particles, by the method described above. An inhibiting factor was demonstrable in all of these sera, with little difference in titer between individual sera.

The inhibition of varying concentrations of tissue suspension by four samples of rabbit serum is shown in Table I. The suspension used here caused ataxia and convulsions in a dilution of 1-160, and no reactions with higher dilutions. With the 1-20 dilution, which represented eight units of tissue suspension, inhibition was produced by each serum in a dilution of 1-4. With two units of suspension the serum titer rose to 1-16, and with one unit to 1-32 in three of the sera.

The length of time required for the inhibition reaction was determined in the following manner. Serial dilutions of rabbit serum were mixed with two units of rabbit brain suspension and placed in a water-bath at 37°C. Samples of the mixture were tested in mice after one minute, and at various intervals thereafter. The results are shown in Table II. No inhibition was demonstrable one minute after preparation of the mixtures. Inhibition was demonstrable in the 1-2 dilution of serum after 5 minutes, in the 1-8 dilution after 30 minutes, and in the 1-16 dilution after 1 hour. No further increase in titer occurred after four hours of incubation.

### *Inhibition of Tissue Suspension from Sources Other than Rabbit Brain*

Several specimens of rabbit serum were tested for their inhibitory action on tissue particles prepared from sources other than rabbit brain, including rabbit lung, mouse brain, human lung, and human brain. In each instance, two units of tissue suspension were employed. The results in all sera were essentially the same, and an illustrative experiment

is shown in Table III. It will be seen that serum produced inhibition of rabbit brain and lung, and mouse brain suspensions, in dilutions of

TABLE I

*Inhibition by Normal Rabbit Sera of the Capacity of Rabbit Brain Tissue Suspensions to Produce Ataxia and Convulsions in Mice*

DILUTION OF TISSUE SUSPENSION†	RABBIT SERUM	DILUTION OF RABBIT SERUM‡					
		1-2	1-4	1-8	1-16	1-32	1-64
1-20	1	0*	0	+	+	+	+
	2	0	0	+	+	+	+
	3	0	0	+	+	+	+
	4	0	0	+	+	+	+
1-80	1	0	0	0	0	+	+
	2	0	0	0	0	+	+
	3	0	0	0	0	+	+
	4	0	0	0	0	+	+
1-160	1	0	0	0	0	0	+
	2	0	0	0	0	0	+
	3	0	0	0	0	+	+
	4	0	0	0	0	0	+

\* 0 = No reaction in mice following injection.

+ = Ataxia and convulsions.

† Dilution figures refer to the final dilution of serum in each mixture.

‡ Dilution figures refer to the final dilution of the sedimentable tissue component, based on the original weight of whole tissue.

TABLE II

*Effect of Time of Incubation on the Inhibition of Rabbit Brain Tissue Particles by Normal Rabbit Serum*

TIME OF INCUBATION AT 37°C.	DILUTION OF SERUM				
	1-2	1-4	1-8	1-16	1-32
1 Min.	+	+	+		
5 Min.	0	+	+		
30 Min.	0	0	0	+	+
60 Min.	0	0	0	0	+
240 Min.	0	0	0	0	+

\* + = Ataxia and convulsions following injection.

0 = No reaction.

1-8 or higher. In contrast, no inhibition of human lung tissue suspension was demonstrable, and only slight inhibition of human brain.

*Thermostability of the Inhibiting Factor in Rabbit Serum*

The effect of heat on the property of serum to inhibit tissue particles was next investigated. Samples of serum were heated for 30 minutes at 56°, 60° and 65°C., and then tested, in two-fold dilutions, for their capacity to inhibit two units of rabbit brain suspension. The results

TABLE III  
*Inhibition by Rabbit Serum of Tissue Particles from Various Organs*

TISSUE SUSPENSION†	DILUTION OF RABBIT SERUM					
	1-2	1-4	1-8	1-16	1-32	1-64
Rabbit Brain.....	0*	0	0	0	+	+
Rabbit Lung.....	0	0	0	+	+	+
Mouse Brain.....	0	0	0	0	0	+
Human Brain.....	0	+	+	+		
Human Lung.....	+	+	+	+		

\* 0 = No reaction following injection.

+ = Ataxia and convulsions.

† Two units of tissue suspension employed in each instance.

TABLE IV  
*Effect of Heat on the Capacity of Rabbit Serum to Inhibit Two Units of Rabbit Brain Tissue Suspension*

TEMPERATURE†	SERUM DILUTION				
	1-2	1-4	1-8	1-16	1-32
Unheated	0*	0	0	0	+
56°C.	0	0	0	0	+
60°C.	0	+	+	+	+
65°C.	+	+	+	+	+

\* 0 = No reaction following injection.

+ = Ataxia and convulsions.

† Rabbit serum heated for 30 minutes at temperature indicated.

are shown in Table IV. Heating at 56°C. did not affect the inhibiting property. At 60°C., the titer was reduced from 1-16 to 1-2. At 65°C. the inhibiting property was eliminated.

*The Effect of Calcium and of Oxalate on the Inhibitor in Serum*

Oxalated plasma from normal rabbits was tested for the inhibition of rabbit brain tissue particles. No inhibition could be demonstrated

in plasma, as is shown in Table V, although serum prepared from the same blood samples exhibited inhibition in a titer of 1-16. Furthermore, the addition of potassium oxalate to serum, in an amount equivalent to that present in oxalated plasma, resulted in the complete disappearance of the inhibitory property of the serum within a few minutes. Equivalent amounts of potassium oxalate in physiological saline or serum produced no symptoms when injected intravenously into mice.

TABLE V

*Comparison of the Capacity of Rabbit Sera and Oxalated Plasma to Inhibit Rabbit Brain Tissue Suspension*

RABBIT	SPECIMEN†	DILUTION OF SERUM OR PLASMA					
		1-2	1-4	1-8	1-16	1-32	1-64
1	Serum	0*	0	0	0	+	+
	Plasma	+	+	+	+	+	+
2	Serum	0	0	0	0	+	+
	Plasma	+	+	+	+	+	+
3	Serum	0	0	0	0	+	+
	Plasma	+	+	+	+	+	+
4	Serum	0	0	0	0	+	+
	Plasma	+	+	+	+	+	+

\* 0 = No reaction following injection.

+ = Ataxia and convulsions.

† Serum and plasma obtained at same bleeding from each rabbit.

The sera which had been inactivated by oxalate could be restored to their original activity by the addition of calcium chloride. Moreover, calcium chloride alone, in small amounts, enhanced the inhibitory property of serum for tissue suspensions. These observations are illustrated in Table VI. The serum used in this experiment had an inhibiting titer of 1-16 against two units of rabbit brain suspension. The addition of 0.1 ml. of 0.1 M potassium oxalate to 0.9 ml. of this serum resulted in the disappearance of the inhibiting property. When calcium chloride was added to the mixtures of oxalated serum and tissue suspension, in an amount sufficient to produce a 0.025 molar concentra-

tion of  $\text{CaCl}_2$ , inhibition was demonstrable in the 1-32 dilution of serum.

Smaller amounts of calcium chloride were then tested for their effect on the inhibiting property of serum in the absence of oxalate. Varying concentrations of calcium chloride were added to mixtures of tissue particles and diluted serum before incubation, and the mixtures were tested after standing for 1 hour at  $37^\circ\text{C}$ . It will be seen in Table VI that the 0.00025 molar concentration of  $\text{CaCl}_2$  was sufficient to cause

TABLE VI

*Effect of Oxalate and Calcium on the Capacity of Rabbit Serum to Inhibit Rabbit Brain Tissue Suspension*

K OXALATE IN SERUM	$\text{CaCl}_2$ IN EACH TUBE	SERUM DILUTION								
		1-2	1-4	1-8	1-16	1-32	1-64	1-128	1-256	1-512
0	0	0*	0	0	0	+	+	+	+	+
0.01 M†	0	+	+	+	+	+	+	+	+	+
0.01 M	0.025 M‡	0	0	0	0	0	+	+	+	+
0	0.025 M	0	0	0	0	0	0	+	+	+
0	0.0025 M	0	0	0	0	0	0	0	+	+
0	0.00025 M	0	0	0	0	0	0	0	+	+
0	0.000025 M	0	0	0	0	+	+	+	+	+
0	0.0000025 M	0	0	0	0	+	+	+	+	+

\* 0 = No reaction following injection.

+ = Ataxia and convulsions.

† Indicates molar concentration of K oxalate in undiluted serum.

‡ Indicates molar concentration of  $\text{CaCl}_2$  in each mixture of diluted serum and tissue suspension.

an increase in the titer of this serum from 1-16 to 1-128. One-tenth of this amount of  $\text{CaCl}_2$  had no enhancing effect.

Calcium chloride alone, in the absence of serum, had no effect on the toxicity of tissue suspensions for mice, when tested under the same conditions and in concentrations similar to those employed in the above experiments.

These observations indicated that the presence of calcium was necessary for the action of the inhibiting factor in rabbit serum. The apparent enhancement of inhibition, which resulted from adding  $\text{CaCl}_2$  to serial dilutions of serum, was interpreted as indicating that the level of effective calcium in normal serum was passed when a serum was

diluted higher than 1-16, while the level of the serum inhibiting factor extended as far as the dilution of 1-128.

The effect of removing calcium from normal serum by dialysis was next investigated. 5.0 ml. of rabbit serum was dialyzed, in a cellophane sac, against physiological saline solution for 48 hours, at 4°C. The dialyzed serum was compared with a sample of the original serum in inhibition tests against 2 units of rabbit brain suspension. The results are shown in Table VII. It will be seen that dialysis resulted in the complete loss of the inhibiting property of serum. The addition

TABLE VII

*Loss of inhibitory Property of Serum after Dialysis, and Restoration of the Property by CaCl<sub>2</sub> and Heat-Inactivated Serum*

SERUM	SERUM DILUTION					
	1-2	1-4	1-8	1-16	1-32	1-64
Untreated.....	0*	0	0	0	+	+
Dialyzed.....	+	+	+	+	+	+
Dialyzed + CaCl <sub>2</sub> †.....	0	0	0	0	+	+
Dialyzed + Heated Serum‡.....	0	0	0	0	+	+
Heated Serum§.....	+	+	+	+	+	+

\* 0 = No reaction following injection.

+ = Ataxia and convulsions.

† 0.025 M CaCl<sub>2</sub> contained in each mixture of dialyzed serum and tissue suspension.

‡ Equal mixtures, in the dilutions indicated, of dialyzed serum and serum heated at 65°C. for 30 minutes.

§ Serum heated at 65°C. for 30 minutes.

of CaCl<sub>2</sub> to the dilutions of dialyzed serum, in a concentration of 0.025 M per tube, caused the restoration of the inhibiting property to its original titer. The inhibiting property of dialyzed serum was also restored by mixing this serum with an equal volume of rabbit serum which had been inactivated by heating at 65°C. for 30 minutes.

It was evident that the factor which was removed from serum by dialysis, and which could be replaced by the addition of calcium chloride, was also contained in serum which had been heated at 65°C. It was therefore of interest to determine whether heated serum could be employed as a diluent for the titration of the inhibitory property in fresh serum. Accordingly, serial dilutions of rabbit serum were pre-



pared in a diluent consisting of a 1-2 dilution of the same serum, which had previously been heated at 65° for 30 minutes. At the same time, comparable dilutions of the fresh serum were made in physiological saline solution. Both rows of diluted serum were then tested for the inhibition of 2 units of rabbit brain suspension. The results are shown in Table VIII. It will be seen that the serum possessed an endpoint of 1-16 when diluted in saline, and an endpoint of 1-128 when diluted in heat-inactivated serum. The latter diluent had no inhibiting effect when tested alone.

In summary, the inhibiting property in rabbit serum was not demonstrable in the presence of oxalate, or in serum which had been dialyzed

TABLE VIII

*Titer of Inhibiting Factor in Rabbit Serum with Heat-Inactivated Rabbit Serum as Diluent, Compared with Titer in Saline Diluent*

DILUENT	SERUM DILUTION†						
	1-8	1-16	1-32	1-64	1-128	1-256	1-512
Heated Serum‡.....	0*	0	0	0	0	+	+
NaCl.....	0	0	+	+	+	+	+

\* 0 = No reaction following injection.

+ = Ataxia and convulsions.

† Serum heated at 65°C. for 30 minutes, and used in a dilution of 1-2.

‡ Figures refer to the final dilution of fresh rabbit serum in the indicated diluent, mixed with two units of rabbit brain tissue suspension.

against physiological saline. The property was restored by the addition of calcium chloride to either the oxalated or the dialyzed serum. It was also restored when dialyzed serum was mixed with serum which had been inactivated by heating at 65°C. When calcium chloride was added to serial dilutions of serum, or when the serum dilutions were made in a diluent of heat-inactivated serum, the titer of the inhibiting property was increased from 1-16 to 1-128. These observations suggest that the inhibition of the toxicity of tissue suspensions for mice is due to the combined action of two factors in rabbit serum, one of which is inactivated by heating at 65°C., and the other a thermostable component which consists, in part if not entirely, of calcium. By themselves, neither of the components causes inhibition of tissue suspensions.

*Fractionation of Rabbit Serum and Identification of Inhibiting Factor in Globulin*

It was next of interest to determine whether the inhibiting factor in serum was present in the albumin or globulin fraction. Accordingly, fractionation of a sample of normal rabbit serum was carried out by precipitation with ammonium sulfate.

9.0 ml. of rabbit serum was diluted with an equal volume of physiological saline solution, and divided into two portions of 9.0 ml. each. 3.0 ml. of saturated ammonium sulfate was added to one portion, and 9.0 ml. to the other, producing, respectively, 25 per cent and 50 per cent saturation. The mixtures were kept at 37°C. for one hour, with occasional shaking, and then centrifuged at 12000 R.P.M. for 15 minutes. The globulin precipitates were each redissolved in 4.5 ml. of physiological saline. The supernatant fluid from the 50 per cent saturated serum was brought to full saturation by the addition of crystals of ammonium sulfate, then kept at 37°C. for 1 hour, and centrifuged at 12000 R.P.M. The resulting albumin precipitate was redissolved in 4.5 ml. of physiological saline. Each fraction was then dialyzed against physiological saline solution for 72 hours, at 4°C. Following dialysis, each fraction was made up to 9 ml. by the addition of saline.

Two-fold dilutions of each fraction were tested for the inhibition of two units of rabbit brain suspension. No inhibitory action was demonstrable in any fraction when physiological saline was used as a diluent. However, since it had been shown that dialysis of whole serum caused the inhibiting property to disappear, and the property could be restored by adding heat-inactivated serum, a 1-2 dilution of serum which had been heated at 65°C. was employed as diluent instead of saline. The results of inhibition tests with this diluent are shown in Table IX. It will be seen that the inhibiting factor was confined to the globulin fraction which was precipitated at 50 per cent saturation with ammonium sulfate. No inhibiting property was demonstrable in the fraction of globulin which was precipitated at 25 per cent saturation, or in the albumin fraction.

*Removal of the Inhibiting Property by Absorption with Tissue Particles*

It was found that the inhibiting property could be partially removed from rabbit serum by absorption with rabbit brain tissue particles. This observation is illustrated by the following experiment.

20.0 ml. of a 10 per cent crude suspension of rabbit brain tissue were centrifuged at 12000 R.P.M. for 30 minutes. The sediment was suspended in 1 ml. of normal rabbit serum. This mixture was placed at 37°C. for two hours, followed by 18 hours at 4°C., after which it was

TABLE IX  
*Distribution of the Inhibiting Factor in Rabbit Serum*

FRACTION OF SERUM†	DILUTION OF FRACTION‡					
	1-4	1-8	1-16	1-32	1-64	1-128
Globulin (25 per cent saturation).....	+	+	+	+	+	+
Globulin (50 per cent saturation).....	0	0	0	0	+	+
Albumin.....	+	+	+	+	+	+
Whole Serum.....	0	0	0	0	0	+

\* + = Ataxia and convulsions following injection.

0 = No reaction.

† Obtained by precipitation with ammonium sulfate.

‡ All dilutions of serum fractions were made in a diluent of rabbit serum which had been heated at 65°C. for 30 minutes.

TABLE X  
*Absorption of Rabbit Serum Inhibitor by Rabbit Brain Suspension*

SERUM	TISSUE SUSPENSION TESTED	DILUTION OF SERUM				
		1-4	1-8	1-16	1-32	1-64
Absorbed with Rabbit Brain Tissue	Rabbit Brain	0*	+	+	+	+
	Mouse Brain	0	+	+	+	+
Unabsorbed Control	Rabbit Brain	0	0	0	0	+
	Mouse Brain	0	0	0	0	+

\* 0 = No reaction following injection.

+ = Ataxia and convulsions.

centrifuged at 12000 R.P.M. for 30 minutes. The supernatant serum was tested for its capacity to inhibit 2 units of rabbit brain suspension, and compared with a sample of untreated serum which had been subjected to the same temperatures and centrifugation. In addition, both sera were tested for the inhibition of 2 units of mouse brain suspension. The results are shown in Table X. It will be seen that the unabsorbed serum possessed an inhibitory titer of 1-16 for both suspensions of brain

tissue. Following absorption with rabbit brain suspension, the end-point was reduced to 1-4, against both suspensions.

Indirect evidence suggesting that an inhibiting factor may have been removed from serum by the tissue particles was obtained in the following manner. The absorption experiment was repeated, using the method described above. After absorption, the tissue particles which were separated from the serum by centrifugation were resuspended in 20 ml. of physiological saline, thus restoring the original 10 per cent concentration. Two-fold dilutions of the suspension were made in saline, and the titer determined by injecting 0.2 ml. of each dilution into mice. At the same time, for comparison, the titer of the original suspension, which had not been exposed to rabbit serum, was determined.

TABLE XI

*Inactivation of Rabbit Brain Tissue Particles by Serum, and Restoration of Activity of Particles by Oxalate*

TISSUE SUSPENSION	DILUTION OF TISSUE SUSPENSION				
	1-10	1-20	1-40	1-80	1-160
Untreated.....	+	+	+	+	0
Serum-treated†.....	0	0	0	0	0
Serum-treated† + Oxalate‡.....	+	+	+	0	0

\* + = Ataxia and convulsions following injection.

0 = No reaction.

† Tissue particles which had previously been incubated with rabbit serum (see text).

‡ Potassium Oxalate 0.01 M.

It was found that the toxicity of the tissue particles used for absorption with rabbit serum had disappeared, even in the 1-10 dilution. In contrast, the original suspension produced ataxia and convulsions in a dilution of 1-80. Potassium oxalate, in a 0.1 M solution, was now added, in the proportion of 1-10, to the tissue suspension which had apparently been inactivated. After several minutes, the titer of this suspension was again determined by the injection of serial dilutions into mice. The results are shown in Table XI. The addition of oxalate caused the reappearance of toxicity, now demonstrable in a dilution of 1-40.

These results indicated that an inhibiting factor in serum was still associated with the tissue particles after removal of the latter from serum, and that the inhibition was reversible by potassium oxalate.

*Attempts to Demonstrate Inhibition of Brain Tissue  
Suspension in vitro*

Evidence has been presented in a previous paper which indicates that the reaction of mice to the injection of tissue suspensions is due to the thromboplastic effect of this material *in vivo*. It is known that tissue particles such as those employed in the present experiments contain thromboplastin, the activity of which can be demonstrated in recalcified plasma. It seemed of importance to determine whether

TABLE XII

*Comparison of the In Vitro Thromboplastic Action and the Production of Reactions in Mice, by Mixtures of Rabbit Brain Tissue Suspensions and Rabbit Serum*

DILUTION OF TISSUE SUSPENSION	TEST	SERUM DILUTIONS							NaCl CONTROL†
		1-2	1-4	1-8	1-16	1-32	1-64	1-128	
1-20	Clotting Time	16"*	15"	15"	14"	13"	15"	15"	17"
	Mouse Injection	0‡	0	0	+	+	+	+	+
1-160	Clotting Time	25"	26"	28"	21"	18"	18"	18"	22"
	Mouse Injection	0	0	0	0	0	+	+	+

\* Figures refer to clotting time of recalcified rabbit plasma, expressed in seconds, produced by the mixtures of tissue suspension and the indicated serum dilutions.

† Tissue suspension mixed with physiological saline instead of serum.

‡ 0 = No reaction in mice following injection.

+ = Ataxia and convulsions.

the inhibiting factor in rabbit serum could be shown to exert a comparable inhibitory effect on the thromboplastic action of tissue suspensions *in vitro*.

Rabbit brain suspensions were tested as thromboplastin by measuring their effect on the clotting time of recalcified rabbit plasma. 0.2 ml. of tissue suspension and 0.2 ml. of 0.025 M  $\text{CaCl}_2$  were added to 0.2 ml. of oxalated rabbit plasma, at 37°C., and the tubes were observed until coagulation first became detectable. All tests were done in triplicate. By this method, the thromboplastic activity of untreated tissue suspensions was compared with that of suspensions which had been previously mixed and incubated for 1 hour with various dilutions of rabbit serum.

It was found that the *in vitro* method provided little if any evidence of inhibition of the thromboplastic action of rabbit brain suspensions,

except when dilute suspensions of the latter were employed. An illustrative experiment is shown in Table XII, in which are shown the clotting times produced by mixtures of tissue suspensions with serial dilutions of normal rabbit serum, compared with the same suspensions mixed with saline. Also, for comparison, the effect of these suspensions on intravenous injection into mice is shown.

It will be seen that the clotting time produced by a 1-20 dilution of rabbit brain suspension was 17 seconds, and no prolongation of clotting time was observed in this suspension after incubation with rabbit serum. In fact, some enhancement of the thromboplastic effect seemed to occur in the dilutions of serum between 1-4 and 1-128. When the same mixtures were injected into mice, complete inhibition of the toxic action of the tissue suspension was demonstrated in the 1-2, 1-4, and 1-8 dilutions of serum.

When the 1-160 dilution of rabbit brain suspension was employed, the clotting time in the control tube was 22 seconds. Clotting times of 25, 26, and 28 seconds, respectively, were produced by the suspension mixed with the three lower dilutions of serum, suggesting slight inhibitory effect by these dilutions. In contrast, the mixtures of tissue suspension and serum showed complete inhibition in the 1-32 dilution of serum, when tested by injection into mice.

These results indicate that inhibition of the thromboplastic activity of the tissue component under study, which is demonstrable by the *in vivo* effect of this material in mice, is accompanied by very slight, if any, detectable inhibition of thromboplastic activity in recalcified plasma by the method used. The apparent disparity between the two tests may be due in part to the extreme sensitivity of recalcified plasma to small amounts of thromboplastin, and the difficulty in detecting significant time differences within a narrow range in this test. However, other factors may be involved. It is known that fresh serum contains some prothrombin, which might interfere in the *in vitro* test for inhibition. It has also been shown by Maltaner (2) that serum exhibits a thromboplastic effect in recalcified plasma, which becomes enhanced in the presence of tissue thromboplastin. Perhaps this is the basis for the slight enhancement of thromboplastic activity which was observed above in some of the mixtures of tissue suspension and rabbit serum.

## DISCUSSION

The property of normal rabbit serum to inhibit the *in vivo* thromboplastic effect of tissue suspensions appears to be due to the combined action of two separate agents. The first of these is a non-dialysable factor, contained within the globulin fraction of serum, and inactivated by heating at 65°C. The second is dialysable, resistant to heating, and consists, in part if not entirely, of calcium. Neither the globulin fraction nor calcium, when used alone, have any demonstrable effect on the tissue component.

The reaction between the serum inhibitor and the tissue component may consist of an actual combination of the two. Evidence which suggests such a combination was obtained in absorption experiments, in which it was found that the titer of serum inhibitor was reduced when the tissue component was added and subsequently removed by centrifugation. Also, the tissue component remained inactive when it was removed from serum and resuspended in saline, but regained almost all of its original activity when oxalate was added. The latter observation indicates that the inhibition reaction is reversible, and may be dependent upon the continued presence of calcium for its maintenance.

Certain analogies may be cited between the inhibition reaction and non-specific complement fixation reactions which are known to occur between normal serum and tissue suspensions. Maltaner (2) has pointed out that a correlation exists between the complement-fixing properties of tissue suspensions and the thromboplastic activity of the same preparations, and has shown that the combination of normal serum and thromboplastic tissue suspensions results in an enhancement of the thromboplastic effect, when the mixtures are added to recalcified plasma. These results are precisely the opposite to the finding of inhibited thromboplastic action which was encountered in the present study by the *in vivo* method. An explanation for the differences between the *in vivo* and *in vitro* reactions is not available. It should be mentioned that in Maltaner's experiments, the mixtures of serum and tissue suspension were tested shortly after being prepared. In the present study, it was found necessary to incubate the mixtures of tissue and serum for 1 hour at 37°C. in order to demonstrate the inhibition reaction.

There are certain similarities between the components which participate in the inhibition reaction, and those which were shown by Kidd and Friedewald (3) to be involved in a complement fixation reaction between normal rabbit serum and mammalian tissue. The tissue component which comprised the antigen in the complement fixation reaction was found by these workers to be entirely sedimentable by high speed centrifugation. The thermostability of centrifuged suspensions was greater than that of crude suspensions, which resembles the observed changes in the thermostability of the tissue component responsible for convulsions and ataxia in mice (1). The serum factor which Kidd and Friedewald described was contained in the globulin fraction, and had the same range of thermostability as the serum factor which is involved in the inhibition reaction. Such similarities are of no more than speculative value, but it is of interest to consider the possibility that the "non-specific" complement fixation reaction and the inhibition reaction described in this paper may be related phenomena.

The actual significance of the inhibiting factor in serum is not known. The uniformity with which it was encountered in all of the rabbits studied, and the constancy of the levels in different sera which were shown by serum titrations, suggest that it may be in some fashion involved in the normal coagulation mechanism. However, there is insufficient evidence to indicate the nature of its role.

The present paper is confined to observations on the inhibiting property of normal rabbit serum. It should be mentioned that a similar factor has been found in other varieties of mammalian serum, including human serum. For practical purposes, the most satisfactory method for determining the amount of inhibiting factor appears to be one which involves the use of heat-inactivated homologous serum as diluent, or in which calcium in excess is added to the higher dilutions of serum tested. By this method, an inhibitor of the sedimentable component of human brain tissue suspensions has been demonstrable in normal human serum, in dilutions as high as 1-128 or 1-256.

#### SUMMARY

The toxic reaction resulting from intravenous injections of a thromboplastic sedimentable component of rabbit brain tissue, in mice, was prevented by previous incubation of the tissue suspension with normal



rabbit serum. The factor in serum which caused inhibition of the tissue component was not affected by heating for 30 minutes at 56°C., was reduced in activity at 60°C., and completely inactivated at 65°C. It was contained within the globulin fraction of serum.

The presence of calcium was necessary for the inhibitory reaction to take place. Oxalated rabbit plasma showed no inhibitory property. The addition of oxalate to serum prevented inhibition from taking place, and the subsequent addition of calcium caused the restoration of inhibitory action. Dialysis of serum against physiological saline removed the inhibitory property, and the addition of calcium restored it. The addition of calcium to diluted serum caused an enhancement of the inhibitory effect. Calcium alone, without serum, had no effect on the toxicity of tissue suspensions.

Serum which had lost its inhibitory property through dialysis could be restored to activity by mixing with serum which had been inactivated by heating at 65°C.

The inhibitory property in serum was partially removed by absorption with the thromboplastic tissue component. The tissue component remained inactive after removal from serum, but regained its activity when oxalate was added.

The available evidence indicates that the serum factor which inhibits the thromboplastic action of brain tissue suspensions is composed of two portions, one a thermolabile globulin component, and the other a thermostable, dialysable component, which is composed, at least in part, of calcium.

#### REFERENCES

1. THOMAS, L.: Bull. Johns Hopkins Hospital 81: 1, 1947.
2. MALTANER, F.: Proc. Soc. Exp. Biol. and Med. 62: 302, 1946.
3. KIDD, J. G., AND FRIEDEWALD, W. F.: J. Exp. Med. 76: 543, 1942.

# POLYMYXIN: A NEW CHEMOTHERAPEUTIC AGENT

P. G. STANSLY, R. G. SHEPHERD AND H. J. WHITE

*Chemotherapy Division, Stamford Research Laboratories of American Cyanamid Company,  
Stamford, Connecticut*

In the course of a program designed to find new antibiotic substances for the chemotherapy of gram-negative bacterial infections, an organism was isolated from soil which produced on an agar plate a wide zone of inhibition of the gram-negative pathogen, *Salmonella schottmuelleri*. The antibiotic-producing organism has been identified as *Bacillus polymyxa* and the antibiotic substance accordingly designated "Polymyxin."

Polymyxin was isolated from the cell-free fermentation liquor as a white, water-soluble hydrochloride. It is unique in its remarkable specificity for gram-negative bacteria thus distinguishing it from all antibiotics hitherto reported. This distinction is further supported by certain chemical and physical properties of the isolated concentrates.

In experimental infections produced by gram-negative bacteria in mice, polymyxin is a highly effective chemotherapeutic agent with a very favorable ratio of therapeutic to acute lethal dose. Of particular interest with respect to chemotherapeutic application is the fact that from a variety of sensitive species, strains resistant to polymyxin have not been obtained under conditions which readily yield strains completely resistant to streptomycin.

The present communication summarizes the main results concerning polymyxin which have been obtained during the course of the past several years. Detailed reports on the various aspects of this work are in preparation.

*Identification of Bacillus polymyxa.* The identification of this organism was aided greatly by following the key to the identification of aerobic spore-forming bacteria by Smith, Gordon and Clark (1).

Briefly, the following observations characterized the antibiotic-producing organism and established its identity as *Bacillus polymyxa*. Broth cultures 18 hours old revealed gram-negative rods with few or no gram-positive cells. Older cultures showed vegetative cells and

oval spores either free or central to terminal in adhering and swollen sporangia. Carbohydrates such as dextrose, lactose and sucrose were fermented with the formation of both acid and gas. Under the conditions recommended for the respective tests (1), the organism produced acetylmethyl carbinol but no amylase that could catalyze the formation of crystalline dextrans from starch (2).

*Potency Assay.* The unit of activity is equivalent to the amount of polymyxin per milliliter in Trypticase Soy nutrient agar (Baltimore Biological Laboratory) which just inhibits the growth of the test organism (*Escherichia coli*, MacLeod strain). A filter paper disc agar diffusion method of assay similar to that commonly used for the assay of penicillin or streptomycin but utilizing the above organism has been devised. Under suitable conditions, the method gives a straight line relationship between log concentration and zone diameter over a range of at least 1 to 256 units per milliliter.

Important factors in the assay are the time and temperature of incubation. Solution-saturated discs are placed on seeded agar plates and incubated for 16–18 hours at 25°C. followed by 6 hours at 37° C. Another factor which increased the sensitivity of the assay and the definition of the inhibition zones was the pH of the antibiotic solutions. As a routine measure assays are performed with solutions at pH 2 in .05 M glycine buffer. Buffer alone at this pH gives no inhibition zone. Standard solutions of the antibiotic in glycine buffer at pH 2 were found to be stable indefinitely in the refrigerator.

The method for estimating potency is essentially that given by Knudsen and Randall for penicillin (3). As routinely performed, the error of the assay is approximately  $\pm 15\%$  for a 95% probability.

*Production.* Polymyxin occurs in the cell-free fermentation liquor after cultivation of *Bacillus polymyxa* for 2 to 5 days at 20–30°C. in a neutral medium consisting of 1% glucose, 2% ammonium sulfate, 0.5% yeast extract, 0.2% potassium dihydrogen phosphate, 0.05% magnesium sulfate heptahydrate, 0.001% ferrous sulfate heptahydrate and 0.005% sodium chloride.

Stationary production is satisfactory in shallow layers, e.g. 100 milliliters per 500-milliliter Erlenmeyer flask. Deep fermentation requires moderate aeration. The rate of aeration used for routine production on a laboratory scale (15 liters of medium per 22-liter

round-bottom flask) was approximately 1 liter of air per minute per 15 liters of medium. One milliliter of 1% octadecanol in mineral oil per liter of medium satisfactorily controlled foaming. Deep fermentation ordinarily yields liquor having a potency of about 200 units per milliliter. The yield from shallow layer production is somewhat less.

*Isolation of the Active Principle.* The clarified 5-day fermentation liquor is treated with a minimum quantity of charcoal (0.4 gram of Darco G-60 per 100 milliliters) to adsorb all the activity. The charcoal is washed successively with water, neutral 50% aqueous ethanol and absolute methanol. The activity is eluted with acidified methanol yielding about 30-50% of the activity of the fermentation liquor. The active principle is then quantitatively precipitated by addition of the eluate to 5 to 10 volumes of acetone. The resulting white powder has a potency of about 1200 units per milligram. Purification of this concentrate through the water-insoluble picrate has resulted in hydrochloride having a potency of about 1800 units per milligram.

*Characteristics of the Isolated Concentrate.* The material obtained as described above is a white, highly water-soluble hydrochloride whose aqueous solutions have a pH of about 5. The aqueous solubility remains high throughout the pH range 1-11. This salt is very soluble in methanol and shows a rapidly decreasing solubility in higher alcohols. It is insoluble in ether, acetone, chlorinated solvents and the hydrocarbons. The activity of polymyxin as determined by the standard assay is relatively stable to heat in aqueous solutions in the pH range 2-7 and in dilute acidic alcohol solutions. However, the activity is destroyed by strong acid or alkali at room temperature in less than 36 hours. The assay titer of a solution of the isolated concentrate is unaffected by prolonged incubation with pepsin, trypsin, pancreatin and erepsin. The activity passes through a cellophane membrane when dialyzed at 10°C. in aqueous solution at pH 7.

Material having a potency of about 1800 units per milligram is an amorphous white powder, is levo-rotatory in aqueous solution and does not give the Sakaguchi reaction. No characteristic absorption in the ultraviolet is observed in the 240-400 m $\mu$  region. The absorption spectrum of this concentrate in the infra-red differs from the spectra of

the known antibiotics such as streptomycin, streptothricin, subtilin, gramicidin-S, tyrocidin and bacitracin.<sup>1</sup>

Preparations of the above potency on treatment in aqueous solution at room temperature with formaldehyde yield a white precipitate upon alkalization. The resulting precipitate on warming with sodium bisulfite dissolves to form a colorless solution. This solution exhibits all the original activity when assayed at pH 7 (the prevailing pH) or by the standard assay procedure after acidification to pH 2. From a solution prepared in this way, the modified active material can be isolated by vacuum freeze-drying as a white, water-soluble, optically-active powder having no melting point. This derivative retains the high order of activity of the hydrochloride both *in vitro* and *in vivo*.

*Antibacterial Activity In Vitro.* When tested by the agar streak plate method (4), using 2% blood infusion agar, 56 out of 64 strains of gram-negative bacteria were found to be sensitive to from 0.5 to 16 micrograms of polymyxin hydrochloride<sup>2</sup> per milliliter of test medium. This group of polymyxin-sensitive organisms included the following species and number of strains: *Aerobacter aerogenes*, 3; *Brucella abortus*, 2; *Eberthella typhosa*, 4; *Escherichia coli*, 3; *Hemophilus influenzae*, 1; *Klebsiella pneumoniae*, 9; *Neisseria intracellularis*, 1; *Pasteurella multocida*, 7; *Proteus vulgaris*,<sup>3</sup> 1; *Pseudomonas aeruginosa*, 3; *Salmonella cholerae suis*, 1; *Salmonella enteritidis*, 2; *Salmonella paratyphi*, 1; *Salmonella pullorum*, 3; *Salmonella schottmuelleri*, 2; *Shigella dysenteriae*, 2; *Shigella paradysenteriae*, 7; *Shigella sonnei*, 2; *Shigella gallinarum*, 1; *Vibrio cholerae*,<sup>3</sup> 1. In a few species, variation in the sensitivity of different strains was observed. Thus, of 8 *Pasteurella multocida* strains tested, 7 were sensitive to 2 and 1 was

<sup>1</sup> We are greatly indebted to Dr. R. C. Gore of the Physics Division for the determination and interpretation of the infra-red spectrum.

<sup>2</sup> Concentrations and doses of polymyxin hydrochloride are expressed on the basis of an arbitrarily assigned potency of 2000 units per milligram for the pure salt. Preparations varying from 200 to 1800 units per milligram gave the same biological response per unit.

<sup>3</sup> *Proteus vulgaris* and *Vibrio cholerae* strains were tested in 2% peptone broth, the *Clostridium* strain in thioglycollate medium and the *Mycobacterium* strain in Dubos medium.

sensitive to 64 micrograms per milliliter. In several cases, very little variation in the sensitivity of different strains was noted. As an example of this, the 11 strains of dysentery bacilli, mentioned above, were all found to be sensitive to from 0.5 to 2 micrograms per milliliter.

Under similar test conditions, 34 strains of gram-positive bacteria were resistant to 64 micrograms of polymyxin per milliliter. These polymyxin-resistant organisms represented the following species and number of strains: *Bacillus mycoides*, 1; *Bacillus subtilis*, 1; *Clostridium welchii*,<sup>2</sup> 1; *Corynebacterium diphtheriae*, 4; *Diplococcus pneumoniae*, 1; *Erysipelothrix rhusiopathiae*, 1; *Mycobacterium tuberculosis*,<sup>3</sup> 1; *Staphylococcus aureus*, 1; *Streptococcus hemolyticus*, 20; *Streptococcus agalactiae*, 1; *Streptococcus faecalis*, 1; *Streptococcus viridans*, 1.

The activity of polymyxin was not affected significantly by variations in the composition of test medium such as the addition of defibrinated rabbit blood up to 50% or by changes in the pH of the medium ranging from pH 5 to 8.

In a study to determine whether resistant organisms could be selected from large populations of cells (10 billion, or more) of each of 15 normally sensitive strains, representing 8 pathogenic gram-negative genera, we have thus far failed to obtain any organisms with an appreciable degree of resistance to polymyxin. Under the same conditions, organisms which for all practical purposes are completely resistant to streptomycin can be readily obtained from most of these strains. Preliminary results of this study are given in Table I.

*Therapeutic Activity.* Polymyxin was found to be highly effective when administered subcutaneously as a single dose to mice infected with virulent strains of *Klebsiella pneumoniae* or *Pasteurella multocida*. In experimental infections produced by each of 5 highly virulent strains of *Klebsiella*, a single dose of 10 micrograms per 20 gram mouse sufficed to protect 80–100% of the animals (Table II). Under similar test conditions, a dose of 40 micrograms was similarly effective in experimental infections produced by each of 5 strains of *Pasteurella* (Table III). Pooled results of several therapeutic experiments using *Klebsiella pneumoniae*, strain BE, and *Pasteurella multocida*, strain 310, are given in Tables IV and V. From these tables it is evident that the Median Survival Doses (S.D.<sub>50</sub>'s) for the *Klebsiella* and the *Pasteurella* infections are approximately 0.4 and 1.0 milligrams per

kilogram, respectively. Polymyxin was found to be equally effective, whether given immediately or 4 hours after infection.

TABLE I

*Selection of Drug-Resistant Bacteria from Large Populations of Normally Sensitive Strains*

TEST ORGANISM AND STRAIN*	AGAR POUR PLATE		NUMBER OF COLONIES† RESISTANT TO	
	Number‡ of Bacteria	Concentra- tion of Antibiotic	Streptomycin	Polymyxin
	Billions	µg./ml.		
<i>A. aerogenes</i> (EF).....	26	160	3	0
<i>A. aerogenes</i> (EF).....	25	1280	11	
<i>E. typhosa</i> (58).....	14	160	220	0
<i>E. coli</i> (MK).....	11	160	7	0
<i>E. coli</i> (MK).....	20	1280	11	
<i>E. coli</i> (MacLeod).....	35	160	2	0
<i>K. pneumoniae</i> (CH).....	19	160	3	0
<i>K. pneumoniae</i> (CH).....	30	1280	5	
<i>K. pneumoniae</i> (CHA).....	22	160	12	0
<i>K. pneumoniae</i> (CHA).....	42	1280	13	
<i>K. pneumoniae</i> (BE).....	18	160	0	0
<i>Paracolon</i> (WT).....	19	160	2	0
<i>P. multocida</i> (310).....	10	160	10	0
<i>P. aeruginosa</i> (CM).....	55	160	81	0
<i>P. aeruginosa</i> (CM).....	26	1280	45	
<i>S. gallinarum</i> (469).....	28	160	0	0
<i>S. paratyphi</i> (Stam.).....	17	160	> 1000	0
<i>S. pullorum</i> (Hol.).....	18	160	0	0
<i>S. schottmuelleri</i> (Merck).....	28	160	3	0
<i>S. flexner</i> (5733).....	17	160	0	0

\* Each of the strains listed above was sensitive to from 2 to 16 micrograms of either streptomycin or polymyxin per milliliter of medium when tested by conventional methods using an inoculum size of approximately 1,000,000 cells. Strains CH, CHA, EF, MK, CM and WT were kindly supplied by Dr. Maxwell Finland.

† Infusion agar pour plates containing each drug were seeded with the indicated number of cells, as determined by colony counts of appropriate dilutions in control plates.

‡ Drug concentrations are expressed as pure base for streptomycin and as pure hydrochloride for polymyxin (see footnote 2).

§ Colonies which appeared on these plates during incubation at 37°C. for 48 hours were subcultured into broth. Relatively small inocula of the strains thus recovered were found to be resistant to 1280 µg. of streptomycin per milliliter.

When the severity of the *Klebsiella* infection was increased by inoculation with 140,000 and with 14,000,000 lethal doses, the dose of polymyxin required for 90–100% survival was 3.2 and 6.4 milligrams per kilogram, respectively (Table VI).

TABLE II

*Therapeutic Activity of Polymyxin Hydrochloride Against Five Strains of Klebsiella pneumoniae in Mice*

*Mice:* Vanderwerken; 16-24 grams.

*Infections:* Intraperitoneal; 0.5 cc. of  $10^{-6}$  dilution of 5-hour broth cultures; 600-1600 lethal doses.

*Treatment:* Subcutaneous; single dose given immediately after infection; aqueous solution of drug at pH 7.0.

DOSAGE*	NUMBER OF MICE PER DOSE	NUMBER OF MICE ALIVE ON 21ST DAY AFTER INFECTION				
		Strain of Klebsiella				
		A-R <sub>n</sub>	A-S <sub>c</sub>	B-B <sub>2</sub>	B-E <sub>G</sub>	C-F
mg./kg.						
4.0	10	10	10	10	10	8
2.0	10	10	10	10	10	9
1.0	10	10	9	10	8	9
0.5	10	10	10	8	10	10
0.25	10	2	8	1	10	6
Untreated	10	0	0	0	0	0

\* Dosage expressed as pure polymyxin hydrochloride. See footnote 2.

TABLE III

*Therapeutic Activity of Polymyxin Hydrochloride Against Five Strains of Pasteurella multocida in Mice*

*Mice:* Vanderwerken; 16-24 grams.

*Infections:* Intraperitoneal; 0.5 cc. of  $10^{-6}$  dilution of broth cultures; 200-800 lethal doses.

*Treatment:* Subcutaneous; single dose given immediately after infection; aqueous solutions of drug at pH 7.0.

DOSAGE*	NUMBER OF MICE PER DOSE	NUMBER OF MICE ALIVE ON 21ST DAY AFTER INFECTION				
		Strain of Pasteurella				
		31	53	221	310	398
mg./kg.						
16	10	10	10	10	10	10
8	10	10	10	10	10	9
4	10	10	10	10	9	10
2	10	8	10	9	8	9
1	10	4	7	6	2	2
Untreated	10	0	0	0	0	0

\* Dosage expressed as pure polymyxin hydrochloride. See footnote 2.



TABLE IV

*Therapeutic Activity of Polymyxin Hydrochloride Against Klebsiella pneumoniae, strain BE, in Mice*

*Mice:* Vanderwerken; 16-24 grams.

*Infection:* Intraperitoneal; 0.5 cc. of  $10^{-6}$  dilution of a 4-hour broth culture;  $5000 \pm 2000$  organisms.

*Treatment:* Subcutaneous; single dose given immediately after infection; aqueous solutions of drug at pH  $7.0 \pm 0.2$ .

DOSAGE*	SURVIVAL—21 DAYS AFTER INFECTION		
mg./kg.	Alive/Total	Per cent	Time†
4.0	70/70	100	
2.0	70/70	100	
1.0	70/70	100	
0.8	76/80	95	4.5
0.6	69/80	86	5.6
0.4	40/80	50	3.8
0.2	0/80	0	1.7
Untreated	0/80	0	1.3

\* Dosage expressed as pure polymyxin hydrochloride. See Footnote 2.

† Average survival time (days) for mice that died.

TABLE V

*Therapeutic Activity of Polymyxin Hydrochloride Against Pasteurella multocida, strain #310, in Mice*

*Mice:* Vanderwerken; 16-24 grams.

*Infection:* Intraperitoneal; 0.5 cc. of  $10^{-6}$  dilution of a 5-hour blood broth culture;  $500 \pm 100$  organisms.

*Treatment:* Subcutaneous; single dose given immediately after infection; aqueous solutions of drug at pH  $7.0 \pm 0.2$ .

DOSAGE*	SURVIVAL—21 DAYS AFTER INFECTION		
mg./kg.	Alive/Total	Per cent	Time†
16.0	30/30	100	
8.0	30/30	100	
4.0	55/60	92	1.8
2.0	43/60	72	1.4
1.0	27/60	45	1.2
0.6	20/60	33	1.1
0.2	0/30	0	1.1
Untreated	0/60	0	1.1

\* Dosage expressed as pure polymyxin hydrochloride. See footnote 2.

† Average survival time (days) for mice that died.

Polymyxin is equally effective whether administered subcutaneously or intravenously; oral administration required about 64 times as much drug to obtain the same therapeutic response (Table VII).

Sixteen milligrams of polymyxin per kilogram of body weight, when administered once daily for 4 days, protected two week old chickens from an otherwise fatal infection with a strain of fowl typhoid (Table VIII).

TABLE VI

*The Influence of Size of Infecting Dose on the Therapeutic Effectiveness of Polymyxin Hydrochloride*

*Organism: Klebsiella pneumoniae; strain B-E.*

*Mice: Vanderwerken; 16-24 grams.*

*Infections: Intraperitoneal; 0.5 cc. of  $10^{-2}$ ,  $10^{-4}$  and  $10^{-6}$  dilutions of a 4-hour broth culture.*

*Treatment: Subcutaneous; single dose immediately after infection; aqueous solutions at pH  $7.0 \pm 0.2$ .*

DOSAGE*	NO. OF MICE PER DOSE	NUMBER OF MICE ALIVE ON 21ST DAY AFTER INFECTION		
		1400†	140,000†	14,000,000†
mg./kg.				
6.4	10			9
3.2	10		9	6
1.6	10	10	8	5
0.8	10	8	2	3
0.4	10	3	0	1
0.2	10	0	0	
0.1	10	0		
Untreated	10	0	0	0

\* Dosage expressed as pure polymyxin hydrochloride. See footnote 2.

† Number of organisms in infecting dose.

**Toxicity.** Polymyxin preparations do not exhibit histamine-like activity. When administered subcutaneously to mice, the L.D.<sub>50</sub> of the hydrochloride<sup>2</sup> was found to be approximately 300 milligrams per kilogram of body weight. Injection of 0.75% solutions of this salt caused local skin irritation. The formaldehyde bisulfite derivative, however, caused no local irritation even when injected as a 15% solution. Furthermore, this derivative was less toxic acutely than the hydrochloride. Preliminary chronic toxicity studies with this derivative have revealed no evidence of toxicity in young rats when

TABLE VII

*Comparative Effectiveness of Polymyxin Hydrochloride Administered by Different Routes*

*Organism: Klebsiella pneumoniae; strain B-E.*

*Mice: Vanderwerken; 16-24 grams.*

*Infection: Intraperitoneal; 0.5 cc. of  $10^{-8}$  dilution of 4-hour broth culture; 5000 organisms.*

*Treatment: Route indicated below; single dose immediately after infection; aqueous solutions at pH  $7.0 \pm 0.2$ .*

DOSAGE*	NO. OF MICE PER DOSE	NUMBER OF MICE ALIVE ON 21ST DAY AFTER INFECTION		
		Oral	Subcutaneous	Intravenous
mg./kg.				
102.4	10	10		
51.2	10	9		
25.6	10	0		
12.8	10	0		
6.4	10	0		
3.2	10	0	10	10
1.6	10		10	10
0.8	10		10	10
0.4	10		9	5
0.2	10		0	0

\* Dosage expressed as pure polymyxin hydrochloride. See footnote 2.

TABLE VIII

*Therapeutic Activity of Polymyxin Hydrochloride in an Experimental Fowl Typhoid Infection*

*Organism: Shigella gallinarum; strain #469.*

*Birds: 2-week old chicks.*

*Infection: Intravenous; 0.25 cc. of  $10^{-1}$  dilution of a 5-hour broth culture.*

*Treatment: Subcutaneous; 0.5 cc. of aqueous solutions of drug; immediately after infection, then once daily for 4 days.*

DOSAGE*	NUMBER OF BIRDS PER DOSE	SURVIVAL—21 DAYS AFTER INFECTION	
		Alive /Total	Survival Time†
mg./kg./day			
16	10	10/10	
8	10	7/10	8.0
4	10	1/10	4.4
Untreated	10	0/10	3.4

\* Dosage expressed as pure polymyxin hydrochloride. See footnote 2.

† Survival time (days) for birds that died.

doses of 100 milligrams per kilogram per day were administered subcutaneously for thirty days.

#### SUMMARY

A new chemotherapeutic agent designated "Polymyxin" has been isolated as the hydrochloride from the fermentation liquor of *Bacillus polymyxa*. The production, isolation and preliminary characterization of this antibiotic have been described and a method for its assay outlined.

Polymyxin is specific for gram-negative bacteria. It is therapeutically active in experimental infections in mice and relatively non-toxic.

From a variety of sensitive species, strains resistant to polymyxin have not been obtained under conditions which readily yield strains completely resistant to streptomycin.

*Acknowledgment:* Valuable contributions to various aspects of the work on polymyxin have been made by the following members of the Chemotherapy Division: isolation of *Bacillus polymyxa* and preliminary characterization of the antibiotic, Miss M. E. Schlosser; identification of *Bacillus polymyxa*, Misses N. H. Ananenko, M. H. Cook and Dr. W. C. Tobie; assay method, Miss M. E. Schlosser; assays, Misses N. H. Ananenko, M. H. Cook and M. E. Schlosser; production and isolation, Misses N. H. Ananenko, M. H. Cook, C. E. Fellows, G. Guillet and M. E. Schlosser; *in vitro* and *in vivo* studies, Miss C. Alverson, Miss M. J. Baker, Mrs. A. H. Clapp, Mrs. M. L. Colborn, Mrs. E. R. Jackson; bacterial resistance studies, Mrs. A. H. Clapp; toxicity studies, Drs. J. T. Litchfield, Jr. and E. J. Robinson and Miss B. C. Wadsworth; purification and chemical studies, Drs. J. P. English and R. Winterbottom, Misses C. E. Fellows, E. B. Leffler and M. M. Rogers.

#### ADDENDUM

Shortly after submission of the present report for publication, a paper reporting the independent discovery of the antibiotic activity of *Bacillus polymyxa* was presented at the the Annual Meeting of the Society of American Bacteriologists, in Philadelphia, on May 16,

1947, by R. G. Benedict and A. F. Langlykke of the Northern Regional Research Laboratory, Peoria, Illinois.

#### REFERENCES

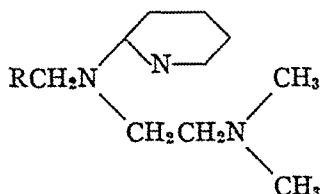
- (1) SMITH, N. R., GORDON, R. E., AND CLARK, F. E.: Aerobic Mesophilic Spore-forming Bacteria. Misc. Publication No. 559, U. S. Dept. Agr., May, 1946.
- (2) TILDEN, E. B. AND HUDSON, C. S.: Preparation and Properties of the Amylases Produced by *Bacillus Macerans* and *Bacillus Polymyxa*. J. Bact., 43: 527, 1942.
- (3) KNUDSEN, L. F. AND RANDALL, W. A.: Penicillin Assay and its Control Chart Analysis. J. Bact., 50: 187, 1945.
- (4) WAKSMAN, S. A., AND REILLY, H. C.: Agar-Streak Method for Assaying Antibiotic Substances. Ind. Eng. Chem., Anal. Ed., 17: 556, 1945.

# 5-HALO-2-THENYL DERIVATIVES OF N,N-DIMETHYL-N'-2-PYRIDYL-ETHYLENEDIAMINE AS ANTIHISTAMINICS

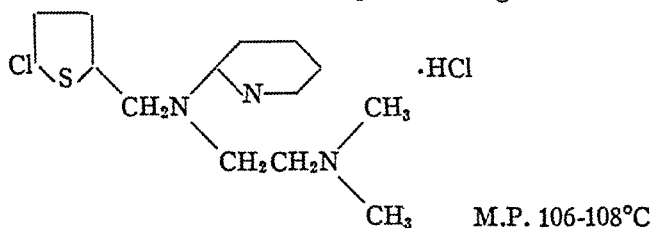
J. T. LITCHFIELD, JR., MAXINE R. ADAMS, LOUISE GODDARD, MARION S. JAEGER AND LILLIAN ALONSO

*Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut*

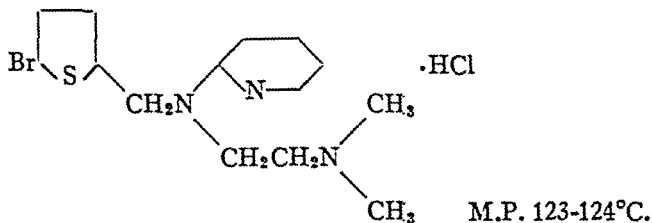
In the course of a study of derivatives of ethylenediamine of the type



where R is a heterocyclic radical, two compounds synthesized in the Chemotherapy Division were found to possess sufficient antihistamine activity to merit extensive investigation. The structural formulae, melting points and names of these two compounds are given below:

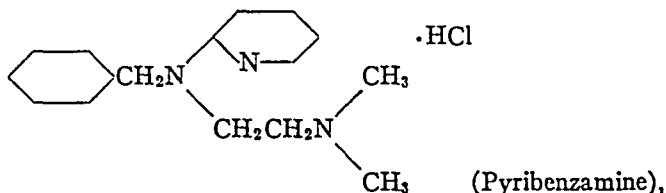


N,N-Dimethyl-N'-(2-pyridyl)-N'-(5-chloro-2-thenyl)-ethylenediamine hydrochloride  
(Chlorothen)



N,N-Dimethyl-N'-(2-pyridyl)-N'-(5-bromo-2-thenyl)-ethylenediamine hydrochloride  
(Bromothen)

The synthesis and chemical properties of this series of compounds will be described in a forthcoming publication (1). For purposes of convenience the two compounds above will be referred to as Chlorothen and Bromothen, respectively. It was felt desirable, in studying these compounds, to evaluate them with reference to an established anti-histamine agent of the ethylenediamine type. For this purpose N,N-dimethyl-N'-(2-pyridyl)-N'-(benzyl)-ethylenediamine hydrochloride,<sup>1</sup>



was selected as a reference standard, since considerable experimental data on its pharmacological properties were available (2-8).

Results of studies made thus far indicate that Chlorothen and Bromothen are twice as active and one half as toxic acutely as Pyribenzamine when compared on a weight basis. When equal doses of the drugs are administered, Chlorothen and Bromothen protect twice as long as Pyribenzamine.

#### METHODS

The experimental techniques used were essentially the same as those reported by other investigators in this field (2-9). In tests utilizing isolated guinea pig ileum, the degree of inhibition of the response to 0.04 micrograms of histamine diphosphate per cc. or 0.02 micrograms of acetylcholine chloride per cc. was studied. When the drugs were examined for activity in guinea pigs, survival or death was found to be the most reliable response.

Histamine aerosols were generated from a 1.25 per cent histamine diphosphate solution. This was found to be nine times the median lethal concentration under our conditions. For intravenous histamine shock, 1 mgm. of histamine (about four times the L.D.<sub>50</sub>) was injected

<sup>1</sup> We are indebted to the Pharmaceutical Group of the Calco Chemical Division of American Cyanamid Company, for supplying this compound for use in these studies.

into the foreleg vein of the guinea pigs. Control mortality rates based on 50 or more animals were 95 per cent for histamine aerosol, and 100 per cent for intravenous histamine. The number of guinea pigs used for each drug in each test summarized in the tables varied from 40 to 180. Dosage values of all drugs used in these studies are given in terms of the salts and not the free bases. Complete details of these and other experiments in progress will be given in subsequent reports.<sup>2</sup>

## RESULTS

Table 1 summarizes the investigations on Chlorothen, Bromothen and Pyribenzamine. In the case of isolated guinea pig ileum, it was found that 5 to 10 minutes exposure to the first two drugs was necessary to develop maximal antihistamine effect<sup>3</sup>. It was found also that the recovery time after exposure to Chlorothen or Bromothen, was 4 to 5 times as long as that for Pyribenzamine. This comparison was made by measuring for each drug, the half-recovery time from equal degrees of inhibition of the histamine stimulus. On the basis of the concentration giving 50 per cent inhibition of histamine Chlorothen and Bromothen were found to be 4 times more active than Pyribenzamine, as is shown by the inhibitory ratios in table 1. However, the three drugs were found to be relatively inactive against acetylcholine.

Using histamine aerosol Chlorothen and Bromothen were compared to Pyribenzamine for activity after oral as well as intraperitoneal administration. In these experiments the percentage of guinea pigs protected (from death) was determined for graded doses of each compound.

<sup>2</sup> We are indebted to Walton Marsh for assistance in standardizing techniques for evaluating antihistamine activity, and to Dr. R. L. Burkhart of the Animal Industry Section of Lederle Laboratories Division of American Cyanamid Company, for furnishing most of the guinea pigs used in these studies.

<sup>3</sup> Chlorothen and Bromothen have physical properties such that they deposit as an oily film especially on any rough glass surface if the pH of 0.01 to 0.1 per cent aqueous solutions is raised above 7.0. Such a film of drug usually cannot be removed by washing with distilled water or Tyrodes solution and may introduce serious error into bioassay and analytical procedures. As a consequence alkaline detergents are not satisfactory for cleaning glassware which has been in contact with these drugs unless the glassware is rinsed also with N/10 HCl and finally with distilled water.



By plotting these data, the Median Protective Dose (P.D.<sub>50</sub>) could be estimated (10). In a similar manner the drugs were compared for protective activity against intravenous histamine. The P.D.<sub>50</sub> values given in table 1 show that Chlorothen and Bromothen are at least twice as active as Pyribenzamine in guinea pigs.

TABLE 1  
*Activity and Toxicity of Antihistaminics*

NATURE OF TEST	DEFINITION OF ENDPOINT		CHLOROTHEN	BROMOTHEN	PYRIBENZ-AMINE	
Isolated Guinea Pig Intestine	<u>Histamine Conc.</u>		100	100	25	
	<u>Inhibitor Conc.</u>					
	<u>Acetylcholine Conc.</u>		0.01 to 0.001	0.01 to 0.001	0.01 to 0.001	
	<u>Inhibitor Conc.</u>					
Lethal Histamine Aerosol	P.D. <sub>50</sub> ± S.E.*	I.P.§	0.22 ± 0.06	0.25 ± 0.04	0.72 ± 0.10	
	mgm./Kgm.	P.O.	2.04 ± 0.45	1.76 ± 0.32	3.54 ± 0.82	
Lethal Intravenous Histamine	P.D. <sub>50</sub> ± S.E.	I.P.	0.10 ± 0.02	0.11 ± 0.02	0.27 ± 0.05	
Lethal Histamine Aerosol	P.T. <sub>50</sub> in hours† after oral dosage		<i>Experiment 1   Experiment 2</i>			
		10 mgm./Kgm.	8.0	7.5	9.0	4.5
		5 mgm./Kgm.	6.2	7.0	5.2	2.1
		2.5 mgm./Kgm.	3.2	3.8	2.6	1.4
		1.25 mgm./Kgm.	2.5	2.5	0.8	<0.5
Acute Toxicity in Mice	L.D. <sub>50</sub> ± S.E.‡	P.O.	390 ± 27	430 ± 25	200 ± 14	
	mgm./Kgm.	I.P.	112 ± 10	130 ± 6	62 ± 7	
Acute Toxicity in Rabbits	L.D. <sub>50</sub> ± S.E.	I.V. ¶	77 ± 14	79 ± 7	27 ± 3	

\* = Median Protective Dose ± Standard Error.

† = Median Protective Time ± Standard Error.

‡ = Median Lethal Dose ± Standard Error.

§ = Intraperitoneal.

|| = Per Os.

¶ = Intravenous.

A comparison of these drugs to Pyribenzamine for duration of protection is summarized also in table 1. In these experiments graded doses of the two compounds were administered by mouth and one hour later the guinea pigs were given the standard exposure to histamine aerosol. The survivors were re-exposed at suitable intervals until all had succumbed. By plotting the cumulative mortality against time and fitting the curve, the Median Protection Time, P.T.<sub>50</sub>, for each

dose of each drug could be estimated. These P.T.<sub>50</sub> values which are given in table 1 were also plotted against the corresponding doses and a straight line fitted for each drug. The ratio: slope constant of a halothen to that of Pyribenzamine is the best estimate of the relative activity of the two drugs, with respect to duration of protection. This ratio was 2.0 and it indicates that when equal doses of the drugs were given, the duration of protection was twice as long for Chlorothen and Bromothen as it was for Pyribenzamine.

TABLE 2  
*Activity and Toxicity of Antihistaminics*

NATURE OF TEST	DEFINITION OF ENDPOINT		THENYL D.P.E.	BROMO- BENZYL D.P.E.	PYRIBENZ- AMINE
Isolated Guinea Pig Intestine	Histamine Conc.				
	Inhibitor Conc.		15	50	25
Lethal Histamine Aerosol‡	P.D. <sub>50</sub> ± S.E.* mgm./Kgm.	I.P.§	1.29 ± 0.21	0.48 ± 0.10	0.35 ± 0.06 0.50 ± 0.10
Lethal Intrave- nous Histamine	P.D. <sub>50</sub> ± S.E. mgm./Kgm.	I.P.	0.43 ± 0.08	0.25 ± 0.04	0.31 ± 0.06
Lethal Histamine Aerosol	P.T. <sub>50</sub> in hours†	2.5 mgm./Kgm.	0.8	1.6	1.4
	after oral dosage	1.25 mgm./Kgm.	<0.5	0.5	0.5
Acute Toxicity Mice	L.D. <sub>50</sub> ± S.E.‡ mgm./Kgm.	I.P.	79 ± 12	160 ± 34	62 ± 7

\* = Medium Protective Dose ± Standard Error.

† = Median Protective Time ± Standard Error.

‡ = Median Lethal Dose ± Standard Error.

§ = Intraperitoneal.

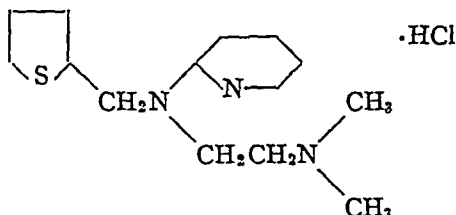
‖ = These results are comparable to results in table 1, only on a relative basis because a different atomizer was used to produce the histamine aerosol.

The results of studies on the acute oral and intraperitoneal toxicity for mice and intravenous toxicity for rabbits are also summarized in table 1, where it can be seen that the halo-thenyl derivatives were one-half as toxic as Pyribenzamine. In all cases in table 1, where standard errors are given, the difference between the value for a halo-thenyl derivative and the corresponding value for Pyribenzamine exceeded the standard error of the difference by more than two-fold and hence could be regarded as significant.

Table 2 summarizes the data obtained on two related compounds

which are of particular interest. The first of these, which is the parent compound of the thiophene series, is:

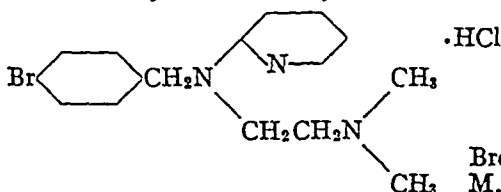
N,N-Dimethyl-N'-(2-pyridyl)-N'-(2-thenyl)-  
ethylenediamine hydrochloride



The synthesis of this compound has been reported independently by Weston (11).

The second one is:

N,N-Dimethyl-N'-(p-bromobenzyl)-N'-(2-pyridyl)-  
ethylenediamine hydrochloride



The data in table 2 show that the thenyl derivative is less than one third as active as Pyribenzamine against histamine aerosol and similarly toxic acutely in mice. Limited studies on the duration of protection indicate that dose for dose Thenyl D.P.E. gives a shorter period of protection than Pyribenzamine (table 2). The data on the Bromobenzyl D.P.E. indicate that its activity and duration of protection are equal to, but its acute toxicity is one-half that of Pyribenzamine. A comparison of Thenyl D.P.E. and Bromobenzyl D.P.E. with Pyribenzamine for activity against intravenous histamine is also given in table 2 and shows that Thenyl D.P.E. is less active than Bromobenzyl D.P.E. and Pyribenzamine.

#### RESULTS OF TOXICITY STUDIES

The studies on acute toxicity of this group of related compounds have been summarized in tables 1 and 2. Although highly significant

differences in toxicity were found, in no case did the symptoms of toxicity differ from one drug to another. All drugs behaved as central nervous system stimulants, causing excitement, tremors, convulsions, tonic and clonic muscle spasms, and respiratory irregularities, all of which have been previously described for Pyribenzamine (3) and Benadryl (12).

Additional studies of the acute toxicity of Bromothen and of Pyribenzamine were made in dogs. A healthy mongrel was given 2 mgm./Kgm. doses of Pyribenzamine intravenously at 20-minute intervals. The second dose precipitated an epileptiform type of convulsion lasting about 5 minutes. Thereafter, the animal exhibited restlessness, respiratory irregularities and frequent aimless movements. A second convulsion occurred after the sixth dose and death occurred after a total of 18 mgm./Kgm. had been administered. A second healthy mongrel was given 4 mgm./Kgm. doses of Bromothen intravenously at 20-minute intervals. After the third dose this animal showed a few signs of beginning toxicity such as occasional aimless movements. After the seventh dose, the animal exhibited excitement, restlessness, irregular respiration, and constant, aimless movements. After receiving a total of 36 mgm./Kgm. a severe epileptiform convulsion occurred followed by unconsciousness. Convulsions recurred intermittently during the next hour, after which the animal became conscious. At 24 hours the animal accepted food and at 48 hours appeared completely normal. This same dog, two weeks later, was given 30 mgm./Kgm. of Pyribenzamine by mouth before feeding. Within 30 minutes a convulsion occurred and this was succeeded by a period of violent excitement. The animal became comatose after the first hour and died 2 hours after the dose was given. A third healthy mongrel survived a single dose of 20 mgm./Kgm. of Pyribenzamine by mouth but not 30 mgm./Kgm. Thus the acute lethal oral dose of Pyribenzamine for dogs is in the range of 20 to 30 mgm./Kgm.

One mongrel and one beagle survived a single dose of 90 mgm. Bromothen per Kgm. by mouth. The mongrel died after 120 mgm./Kgm., but the beagle survived. A second beagle died after 120 mgm./Kgm. Thus the acute lethal oral dose of Bromothen is in the range of 90 to 120 mgm./Kgm.

Two male and two female purebred beagles were given 30 mgm./

Kgm. of Bromothen by mouth daily before feeding. About  $\frac{1}{2}$  hour after the fifth dose the animals showed mild psychosomatic symptoms of short duration, such as were described for Benadryl (12), and which we have noted also with mildly intoxicating doses of Pyribenzamine. These symptoms recurred and seemed to increase in severity and duration with the sixth and seventh dose. Accordingly, the daily dose was reduced to 20 mgm./Kgm. At this level no significant symptoms were encountered up to 30 days. Salivation after dosage was noted in all four dogs. Vomiting occurred twice in the female pair. Both males developed a conditioned salivary reflex about the 25th day of dosage. After 30 days the two females showed some loss of appetite. Accordingly their daily dose was reduced to 15 mgm./Kgm. The four animals eat and behave normally, and there has been no loss of body weight of any consequence during the first 60 days of drug administration. Hematological studies of the dogs were carried out before therapy and after 15 days. No departure from normal values was found.

Five albino mice (Tumblebrook Farms) ingesting 20 mgm./Kgm./day and ten ingesting 160 mgm./Kgm./day of Bromothen gained weight at the same rate as control mice and appeared normal throughout a 30-day period. Ten weanling rats (Tumblebrook Farms hooded strain) ingesting 65 mgm./Kgm./day and ten ingesting 700 mgm./Kgm./day of Bromothen appeared normal throughout a 30-day period. The growth rate of the first group did not differ from that of the controls. The growth rate of the 700 mgm./Kgm./day rats was depressed, as was their daily food intake, so that by the end of the 30 days their body weight averaged 30 per cent less than the controls. The mice and rats were autopsied at the end of 30 days. No gross pathology was found in any of the animals. Microscopic studies of the tissues will be reported subsequently.<sup>4</sup>

#### DISCUSSION

It is well known that thiophene is nearly equivalent chemically to benzene and several thiophene analogs of benzene derivatives have been examined for biological activity (13). In Weston's report on the thenyl

<sup>4</sup> We are indebted to Dr. E. Mayer for pathological examination of the experimental animals.

analog of Pyribenzamine (11) the activity and toxicity were stated to be of the order of Pyribenzamine.

The data reported in this communication indicate that the thenyl analog is neither more active nor less toxic than Pyribenzamine and suggest the compound to be somewhat less active.

Halogenation of the para position of the benzyl group of Pyribenzamine appears to decrease toxicity without significantly affecting activity. On the other hand, introduction of a methoxy group in the para position definitely enhances activity but does not decrease toxicity (9). The effect of halogen in the case of Bromothen and Chlorothen is truly remarkable, since activity is increased four-fold and acute toxicity halved over that of the non-halogenated compound. The acute and chronic toxicity studies of Bromothen in dogs indicate that this compound is well tolerated in doses greatly exceeding those presumably necessary for therapy in man. The long duration of action of Chlorothen and Bromothen suggests that one dose per day may be sufficient in most cases to protect from histamine-type reactions which are encountered in various allergic conditions. However, clinical studies of Chlorothen and Bromothen will be necessary to establish the value of these new antihistaminics.

#### SUMMARY

Four ethylenediamine derivatives were studied for antihistamine activity in comparison with Pyribenzamine, N,N-Dimethyl-N'-benzyl-N'-(2-pyridyl)-ethylenediamine hydrochloride. It was found that replacement of the benzyl by a thenyl group decreased activity without changing toxicity. Replacement of benzyl by 5-bromo-2-thenyl or 5-chloro-2-thenyl increased activity and decreased toxicity. Replacement of benzyl by para-bromobenzyl decreased toxicity but did not significantly change the activity. The bromothenyl derivative of dimethyl-pyridyl-ethylenediamine was found to be well tolerated over a 30-day period by mice and rats and a 60-day period by dogs, in doses greatly exceeding those showing antihistamine activity in guinea pigs.

#### REFERENCES

- (1) CLAPP, CLARK, VAUGHAN, ENGLISH AND ANDERSON: J. Am. Chem. Soc., 69: 1549, 1947.

- (2) MAYER: J. Allergy, 17: 153, 1946.
- (3) MAYER, HAYS, BROUSSEAU, MATHIESON, RENNICK AND YONKMAN: J. Lab. and Clin. Med., 31: 749, 1946.
- (4) YONKMAN, CHESSE, MATHIESON AND HANSEN: J. Pharm. & Exper. Therap., 87: 256, 1946.
- (5) MAYER AND BROUSSEAU: Proc. Soc. Exper. Biol. & Med., 63: 187, 1946.
- (6) YONKMAN, OPPENHEIMER, RENNICK AND PELLET: J. Pharm. & Exper. Therap., 89: 31, 1947.
- (7) MARSH AND DAVIS: J. Pharm. & Exper. Therap., 89: 234, 1947.
- (8) SHERROD, LOEW AND SCHLOEMER: J. Pharm. & Exper. Therap., 89: 247, 1947.
- (9) LOEW, KAISER AND MOORE: J. Pharm. & Exper. Therap., 83: 120, 1945.
- (10) LITCHFIELD AND FERTIG: Bull. Johns Hopkins Hosp., 69: 276, 1941.
- (11) WESTON: J. Am. Chem. Soc., 69: 980, (April) 1947.
- (12) GRUHZIT AND FISKEN: J. Pharm. & Exper. Therap., 89: 227, 1947.
- (13) STEINKOPF: Die Chemie des Thiophens. Reprint Edition published by Edwards Bros., Inc., Ann Arbor, Mich., 1944.

# PODOPHYLLOTOXIN

## A Preliminary Note

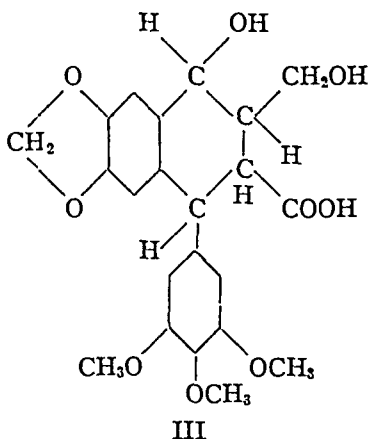
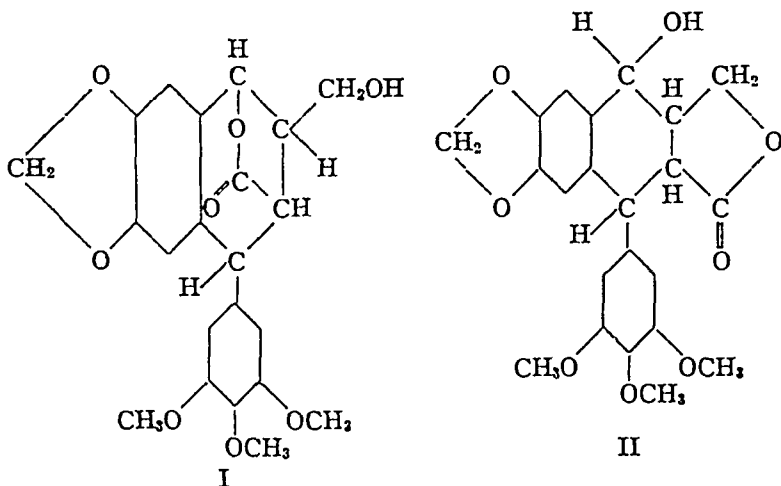
MAURICE SULLIVAN AND KENNETH BLANCHARD

*From the Department of Medicine (Dermatology) and the Department of Pharmacology*

When podophyllin, the resin of *podophyllum peltatum*, is applied to condylomata acuminata there is rapid involution of the tumors (1). Histologic studies of human and rabbit skin, condylomata acuminata and verrucae vulgares treated with podophyllin revealed severe cytotoxic effects and distorted mitotic figures similar to those produced by colchicine (2). Furthermore, condylomata acuminata treated with colchicine were rapidly cured. Podophyllin in powder form or made into a paste with water or incorporated in ointments or suspended in oil or dissolved in alcohol produced the aforementioned cytologic changes. However, when podophyllin was dissolved in normal solution of potassium or sodium hydroxide it proved to be biologically inert (3). This indicated that podophyllotoxin was probably the ingredient responsible for the involution of the tumors and the cellular alterations because podophyllotoxin is easily susceptible to alkaline hydrolysis. Podophyllin is a mixture of four components: podophyllotoxin, picropodophyllin, podophylloresin and quercetin (4, 5). In dilute solutions of both inorganic and organic bases podophyllotoxin (I) rapidly undergoes rearrangement to the isomeric picropodophyllin (II). More concentrated solutions of inorganic bases convert both of these substances to podophyllic acid (III). The structures of the substances shown below, and the nature of these transformations have been established by the investigations of Borsche (6) and of Spath (7). An alternative structure for podophyllotoxin has been suggested by Robertson and Waters (8). This no longer merits consideration since a substance of this structure upon rearrangement to picropodophyllin and subsequent successive dehydration and dehydrogenation should yield a lactone of established constitution (9) which is isomeric with the lactone obtained by the analogous degradation of podophyllotoxin. Taken together with the observation that alkaline solutions of podophyllin appear devoid of action on the skin, these chemical considerations suggest that this is uniquely determined by the podophyllotoxin content which is stated (5, 10) to account for from 28.2 to 50 per cent of commercial



podophyllin. Accordingly, attention was turned to an examination of podophyllotoxin. Podophyllotoxin applied to normal skin and mucous membrane produced gross and microscopic changes identical with



those produced by podophyllin. *Condylomata acuminata* treated with podophyllotoxin rapidly underwent involution.

#### CONCLUSION

Podophyllotoxin appears to be the ingredient in podophyllin responsible for its cytologic effects.

We are indebted to Doctor Milton Bush for a commercial preparation of podophyllotoxin.

## BIBLIOGRAPHY

1. KAPLAN, I.: *New Orleans Med. & Surg. J.* 94: 338, 1942.
2. KING, L. S., AND SULLIVAN, M.: *Science* 104: 244, 1946.
3. SULLIVAN, M., AND KING, L. S.: *Arch. Derm. and Syph.* (In press).
4. U. S. Dispensatory. XX, p. 879.
5. VIEHOEVER, A., AND MACK, H.: *J. Am. Pharm. Assoc.* 27: 632, 1938.
6. BORSCHIE, W., AND NIEMANN, J.: *Ann.* 499: 59, 1932.
7. SPATH, E., WESSELY, F., AND NADLER, E.: *Ber.* 66: 125, 1933.
8. ROBERTSON, A., AND WATERS, R. B.: *J. Chem. Soc.* 83, 1933.
9. HAWORTH, R. D., RICHARDSON, T., AND SHELDRIC, G.: *J. Chem. Soc.* 1576-1581, 1935.
10. WARREN, L. E.: *J. Assoc. Official Agr. Chem.* 13: 117, 1930.

# PROCEEDINGS OF THE MEETINGS OF THE JOHNS HOPKINS MEDICAL SOCIETY

HELD IN HURD MEMORIAL HALL, JANUARY 13, 1947

*The Mechanism of Excretion of Ammonium Thiosulfate.* DR. JOHN FRANKLIN  
and DR. JACQUES GENEST (*Department of Medicine, Johns Hopkins  
Hospital*).

Since the work of Gilman et al. in dogs and Newman et al. in man on the mechanism of excretion of the thiosulfate ion by the kidney showed that the thiosulfate ion is filtered by the glomerulus and not secreted or reabsorbed by the renal tubule, we have determined the fate of intravenously and orally administered ammonium thiosulfate in dogs. As it is an obligatorily excreted anion, we were interested in its effect on the pattern of electrolyte excretion, particularly in reference to the excretion of fixed base.

In dogs the intravenous administration of ammonium thiosulfate for short periods resulted in diuresis and rapid loss of sodium in the urine. Thiosulfate was recovered in the urine partly as sulfate (20-30 percent) and mainly as thiosulfate (70-80 percent). Oral administration of thiosulfate led to recovery of one-half as thiosulfate and one-half as sulfate in the urine. The loss of sodium was not so marked as from the intravenous administration due to the increased production of ammonia.

The continuous intravenous administration of ammonium thiosulfate resulted in excessive sodium excretion, renal failure and death with a serum electrolyte picture similar to that of Addison's disease; a very low serum sodium (125 Meq/Lt) and a high serum potassium (12 Meq/Lt). Analysis of the water content of this dog's muscle showed an increase to 89 percent water above a normal of 76 percent.

The potentiality of the drug as a diuretic and as a tool for interpreting the intricacies of the excretion pattern of electrolytes in clinical problems of salt and water balance was suggested.

## DISCUSSION

*Dr. Ephraim Shorr:* What do you regard as the mechanism of the renal failure observed after continuous intravenous administration of ammonium thiosulfate?

*Dr. John Franklin:* We have no direct evidence as to the cause, but we feel that it was secondary to the circulatory failure associated with what we regard as terminal potassium intoxication. Darrow, Finch and others have demonstrated the serious cardiac effects of potassium poisoning. Cardiac standstill has been recognized as the immediate cause of death.

*Dr. James Bordley:* I would like to ask if you know exactly where the thio-sulfate is converted to sulfate; whether that event occurs in the kidney or whether it occurs elsewhere in the body, because I should think the amount of sodium which is removed to some extent depends upon the place where that conversion occurred.

*Dr. Franklin:* I cannot answer that question, Dr. Bordley, since we have not studied that specific problem. Gilman has studied the volume distribution of thiosulfate and has observed that oxidation occurred for the most part during and immediately following injection. We feel that by the oral route, a major portion of the conversion takes place in the gastrointestinal tract. Sulfate excretion in the oral experiment accounted for approximately 50 percent of the recovered drug. We have extended Gilman's work by showing that the increased sulfate excretion accounts for that portion of the administered drug not recovered as thiosulfate. Gilman recovered some 70 to 80 percent of the administered drug, but did not account for the remaining 20 to 30 percent.

*Dr. Richard Bing:* I would like to ask whether you have seen any changes in renal plasma flow? It has been said that sodium thiosulfate does produce an increase in renal plasma flow and I wondered if you have seen similar changes in these experiments?

*Dr. Franklin:* We have not determined renal plasma flow in these experiments. However, the constancy of the thiosulfate clearance in humans despite high serum concentration after the single injection technique suggests that there was no significant vasodilatory effect.

*Dr. John Lutscher:* The chart of blood changes after intravenous ammonium thiosulfate showed a terminal fall in total serum proteins. I wonder if you have any comment on the fact that this is quite different from the usual rise in protein concentration in acute and chronic dehydration in man and experimental animals.

*Dr. Franklin:* I did not comment on that because we do not have a good explanation. There is one observation that can be made. The fall in total serum proteins occurred during the period of renal failure when the dog was in positive fluid balance. We can surmise that one of two things occurred. First, that the limit of transfer of water into the cell had been reached, or secondly, that in the face of decreased fluid output and continued infusion of ammonium thiosulfate, serum dilution was present. If you noticed, there was also a terminal fall in the hematocrit from 47 to 42 percent. I have no further explanation. Perhaps you have.

*Dr. Lutscher:* Did Dr. Zierler find any evidence of a transfer of protein into the cells?

*Dr. Franklin:* No, only the transfer of water into the cell was measured.

*Dr. Shorr:* I wonder whether the fall in blood volume was of sufficient magnitude to provide an explanation for renal failure similar to what which occurs in circulatory collapse from peripheral circulatory failure and secondary shock?

*Dr. Franklin:* I should imagine that decreased plasma volume played a role in the renal failure. We felt that potassium intoxication played the major role, however, because of the rapidity of exitus of this dog. Terminally, there was evidence of some serum dilution.

*Hepatorenal Factors in Traumatic and Hemorrhagic Shock.* DR. EPHRAIM SHORR (Cornell University Medical College, New York, N. Y.).

Dr. Shorr described his technique of assaying vaso-excitor and vaso-depressor substances in the blood during various types of shock. The part played by the liver and kidneys in the formation and elimination of these materials was discussed. Failure of the normal mechanisms during prolonged shock may account for the "Irreversibility" of the late stages of shock.

#### DISCUSSION

*Dr. James Bordley:* Dr. Shorr, I wasn't quite clear on one point. You spoke of the relative oxygen requirements of the kidney and the liver as possibly explaining the fact that the kidney factor acted before the liver factor. Is the same thing true in vitro? Does the kidney factor appear at a lower oxygen tension than the liver factor?

*Dr. Shorr:* The kidney factor appears more readily than the liver factor. Of course, when we are dealing with slices in vitro I think we have to remember that we set up semi-artificial conditions. For example, the effect of a given concentration of oxygen will depend on the thickness of the tissue, so that kidney slices must be very thin. Liver slices can be thicker, and for that reasons it is a little difficult to be as arbitrary about the relative effects of reductions in oxygen tension on the appearance of the vaso-excitor factor. If we were to cut our kidney slices half as thin as we ordinarily do, then a greater reduction in oxygen tension would be required to produce a similar degree of anoxia, so that I think it is a little unsafe in view of the fact that we are dealing with the process of diffusion to be too dogmatic about relative reductions in oxygen tension which might be required by the kidney as compared to the liver.

*Dr. Alfred Blalock:* I have enjoyed this presentation by Dr. Shorr on this very important problem. He referred at the beginning of his paper to some of the earlier work that had been done on so-called toxic substances. As a result of the work of Cannon, Bayliss and others it was generally assumed shortly following the

first World War that the most important initiating factor in traumatic shock was the absorption of toxic products from an injured area. Studies in the 1930's cast a good deal of doubt on the results and the interpretations of results that had been given earlier. These studies showed that the local and regional loss of whole blood or plasma or both is the most important initiating factor in the vast majority of cases of traumatic shock. The liberal use of blood and plasma reduced tremendously the number of instances of shock that is seen in surgical patients. Unfortunately we still see an occasional patient who develops shock and who does not respond well to therapy. I think that such cases are seen more often on a medical service than on a surgical service. In fact, much remains to be learned about the pathogenesis of shock in many so-called medical disorders.

It is my impression that the pathogenesis of some instances of surgical or traumatic shock is fairly well understood if we limit the consideration to the early or so-called reversible stage. The question becomes much more complicated when we deal with the advanced or so-called irreversible stage. Many alterations have occurred at that time and there are probably many factors at work in causing the "irreversibility". It is on this stage of shock that Dr. Shorr's contributions are particularly valuable.

I think that one might raise a good many questions regarding the findings which Dr. Shorr has reported. For example, one might question the interpretation of results which are obtained by observing the capillaries of the meso-appendix. Even if it is agreed that one material is "vaso-depressor" and another is "vaso-excitor", there is no proof that the former is the cause of shock. The question can be raised as to whether the blood vessels of the rat's meso-appendix are a good indication of the reaction of the blood vessels of the rest of the body. After all, one is looking at only a few capillaries in a restricted area of the body.

Dr. Shorr reported experiments in which a tourniquet was applied to an extremity and this extremity was subsequently placed in a cast. The statement was made that no fluid could be lost into the extremity after the tourniquet was removed. It is true that a great quantity of fluid cannot be lost but I would suspect that there is some loss.

In two of the last slides which were shown by Dr. Shorr you will have noted that one of the initial features of the experiment was the removal of blood equal to  $2\frac{1}{2}$  to 3% of the body weight of the animal. Loss of blood in this amount will in some animals by itself alone cause shock and should certainly be taken into account when one is adding this procedure to some other method of causing shock.

The clinician should always keep in mind the maintaining of the blood volume at as nearly the normal level as possible. In this manner the vast majority of cases of shock can be prevented. This statement, however, is in no way meant to minimize the importance of studies such as Dr. Shorr has reported on the advanced stages of shock. It is to be hoped that such studies will result in one's being able to alter what is now regarded as irreversible shock to the type that can be effectively treated.

*Dr. Shorr:* I appreciate your comments, Dr. Blalock. First of all, as to the test. It is not something that is learned very rapidly. We take about six weeks to two months to train our technicians and work entirely by a code system. It has been this method, for example, by which out of a crude liver extract a material with consistent vaso-depressor activity has been fractionated, so that it had to be reasonably reliable to permit us to make that fractionation. I feel that objections to the test require us to provide, if possible, other pharmacological tests for the same material; and this we are attempting to do, so that it will be possible to duplicate the work without quite the same amount of apprenticeship to a difficult method.

Now as to the induction of shock in the animals whose limbs are taped, what I meant to say was that the amount of fluid loss in those limbs was so small as not under ordinary circumstances to produce shock. Actually those limbs were dry. However, in taping, unless casts are placed and even in the presence of casts, it is possible to lose a moderate amount of fluid, but I think that the extent of the fluid loss is much too small to be responsible for this sequence of events which leads to the profound and final circulatory failure. We have worried about just where the fluid goes and I think some experiments in eviscerated rabbits are helping us, as well as experiments in animals on low protein diets who have lost their inactivating system. I have a high respect for the capacity of the liver to take up blood. Shock can be induced in an eviscerated animal with the kidneys tied off, the liver intact, the portal vein tied off, and the hepatic artery furnishing the only supply to the liver; ligate the hepatic artery for a matter of 60 to 90 minutes for the formation of the vaso-depressor material, release it at the end of that time and flood the circulation with vaso-depressor material and the animal goes into circulatory collapse. Without the liver in those animals injection with vaso-depressor material will not cause them to go into shock. Our experiments would indicate that the liver is a very likely organ for pooling of blood and once the liver vessels are dominated by vaso-depressor material their sphincter arrangement is such as to allow them to pool just as well as the mesentery. While this is to some degree inferential, the congestion of the liver that is seen in late shock indicates that it does indeed take up blood. Now when one releases the tourniquet from the limb that has been placed in the cast and into which very little blood loss has taken place, one finds that the first thing one can observe is the release of the vaso-depressor material in quantities from those limbs and then it is taken up very quickly in the liver. We picked it up at the time when the liver inactivating system was entirely intact; whereas we know that if the liver had formed as much vaso-depressor material as that, the liver inactivating system would have been severely damaged. The liver had taken up the vaso-depressor material, which has led to pooling of blood in the liver and the reduction of blood volume.

I would like to point out what I think I may not have emphasized—that we believe that the fundamental factor which makes against recovery is the damage to the inactivating system. Unfortunately, shock of this character is associated with

renal impairment so that the renal excretory mechanism is of no help. But if the hepatic inactivating system is helped to liberate the blood stream of the vaso-depressor material then transfusion should take hold. It may very well be that the management of shock through massive transfusions of whole blood which were given the latter part of the war may have achieved success by allowing sufficient time for survival of the patient during which there could be a regeneration of the liver enzyme system.

FEBRUARY 17, 1947

*The Implications of Rh Incompatibility in Transfusion of Women.* DR. LOUIS M. HELLMAN (*Department of Obstetrics*).

Nine Rh-negative women are presented who have received transfusions and who subsequently delivered children with erythroblastosis or developed antibodies which endangered their future childbearing careers. These nine women had five normal children prior to their transfusions and but two following. Both of the normal children after transfusion were Rh-negative and these women had heterozygous husbands. The patients with homozygous mates had no normal children after transfusion, and of the eight born to them, the expected survival rate of 50 percent occurred. The future childbearing of all of these women has been seriously jeopardized and particularly those with homozygous husbands. At least one of these latter women has requested and received sterilization for this reason.

It might well be argued that all but three of these patients had one or more pregnancies prior to the transfusion and that the pregnancy alone might well be sufficient to immunize them. Certainly this fact cannot be denied but it is interesting to note that a history of previous transfusion occurs in but two percent of our patients, while in the twenty-seven instances of erythroblastosis a history of transfusion was present in seven women or twenty-six percent. It is difficult to feel that transfusion does not play an important role in the production of erythroblastosis. With the increased use of transfusion the chances of unwittingly administering Rh-positive blood to Rh-negative females has definitely increased. It would appear that a high degree of negligence is present if transfusion is ever given to a female child or women in the childbearing age without first determining the Rh-compatibility. This is certainly the very minimum care that can be expected. For some time now no transfusion has been given in the Johns Hopkins Hospital to individuals of either sex without first determining the Rh-compatibility, except in cases of dire emergencies and then Rh-negative blood is always given.

Conclusions: Nine Rh-negative women are presented who gave a history of transfusion.

2. The probable damage to their future childbearing careers is pointed out.

3. The necessity of comparing Rh-compatibility prior to the transfusion of females is emphasized.



## DISCUSSION

*Dr. Eastman:* I should like to make a few remarks supplementing and re-emphasizing what Dr. Hellman has said about the role which transfusions play in causing stillbirths and neonatal deaths. We are becoming increasingly impressed by the role that transfusions rather than repeated childbirths play in bringing about this entity. An Rh-negative woman married to an Rh-positive man can have baby after baby sometimes without any dire results because occasionally the Rh-positive cells do not find their way through the placenta and for this or other reasons she is not immunized. But when we have an Rh-negative mother married to an Rh-positive husband, and let there be a history of transfusion, we feel that the chances are about nine out of ten that she will get into difficulty and will have been rendered unfit for childbirth. Now Dr. Hellman has referred to the fact that after all this disease doesn't occur very often and perhaps we have exaggerated the importance of it. A week or so ago at one of the medical clinics I referred to the fact that today erythroblastosis is a commoner cause of stillbirth and neonatal death than syphilis. One or two of the group later raised some question about this and I looked it up and found that in the City of Chicago in the year 1944 the stillbirths and neonatal deaths due to erythroblastosis exceeded those due to syphilis. These tragedies which we see so frequently cause a great deal of heartache and mental anguish and I would go much further than Dr. Hellman and beg of you in the practice of medicine, surgery and pediatrics or any others who have to do with the transfusing of females, whether they be a few hours old or whether they be 45 years old, to consider that the chances are in the order of one to seven or eight that the transfusion is Rh incompatible. Accordingly, I urge you in these cases to do Rh cross-matching so that we here will be in a position to say that we are not responsible for this accident having occurred.

*Dr. Janet Hardy:* I would like to emphasize the importance of Dr. Hellman's paper from the point of view of the pediatrician. One cannot help being impressed by two things. Firstly, that these erythroblastotic babies whose mothers were sensitized by blood transfusions, tend to be terribly sick and present complicated problems of medical care; 50% of them die. Secondly, the majority of infants seen here with erythroblastosis during the past two years have been born to mothers sensitized by blood transfusions. Few have been born to mothers sensitized by previous pregnancies and these have all been mild, presenting no serious problems.

*Physiological Studies in Congenital Heart Disease.* DR. RICHARD BING, DR. L. R. VANDAM and DR. FRANK GRAY (*Department of Surgery*).

Dr. Bing's reports were published in the February and June issues of the BULLETIN.

## DISCUSSION

*Dr. James Bordley:* I am sure that Dr. Bing is fully aware of the questionable validity of the assumptions upon which some of his conclusions are based. He and his associates deserve a great deal of credit for having undertaken this formidable job in spite of the theoretical objections which could be raised. They have had opportunity to make observations on an astonishingly large group of individuals suffering from what would usually be considered a rare cardiac disturbance. The correctness of many of their deductions has been verified by subsequent surgical intervention, and by repetition of observations after operations. Their experience clearly indicates that in spite of the questionable assumptions and theoretical objections, information can be obtained by their methods, which is of real practical value in deciding whether to operate on a patient who has multiple congenital cardiac defects. Quite apart from the findings in this particular group of cases, it should be pointed out that these methods can give practical assistance in arriving at an accurate diagnosis in less complicated types of cardiac defects. This was recently demonstrated to me in the case of a patient whom I referred to Dr. Bing. This young woman, aged 32 years, had led a perfectly normal life until the age of 24 when in the midst of a pregnancy she was found to have a loud cardiac murmur. Since then she had been treating herself as an invalid though she had few specific symptoms referable to the heart. On physical examination, the heart was not enlarged but there was a loud murmur over the upper left chest and back, quite typical of the murmur of a patent ductus. Fluoroscopic and x-ray studies showed that the heart and great vessels were normal in size and there was no undue prominence of the conus or pulmonary vessels. We could not be certain that the patient had a large enough shunt from aorta to pulmonary artery to cause the vague symptoms of which she complained. Nor could we be certain that we were dealing with an uncomplicated patent ductus. Dr. Bing and his associates calculated from their observations that the output of the left ventricle was about twice that of the right ventricle. They found no evidence of any other cardiac defect. The conclusion was that the patient had a large shunt through the ductus, and this proved to be the case at operation. Dr. Blalock found that there was a patent ductus measuring more than one centimeter in diameter. The ductus was closed and all of the signs over the heart disappeared. Two months after operation the patient is now making very satisfactory progress. In this case, Dr. Bing's findings afforded great practical assistance in the proper evaluation of the patient's symptoms and gave us much greater confidence in discussing prognosis and treatment with the patient and her family.

*Dr. Joseph Lilienthal:* I think it is probably fair to say that Dr. Bing and his colleagues have had a unique experience in their opportunity to study so large a group of individuals who have been exposed to chronic anoxemia for months and years. Furthermore, they have had an opportunity to study them while the investigators themselves were under normal conditions in contrast to the studies

carried out at high altitude. For these reasons it would be of interest to learn what data have been observed which may throw light on the adjustments which these patients have made in response to chronic anoxemia. Dr. Bing intimated that there was a change in basal oxygen consumption. Would he be good enough to amplify on this interesting observation and any other data of this nature?

*Dr. Bing:* We don't have many data on the subject yet. A dissociation curve is being constructed in this laboratory by Dr. Spencer with the method of Dr. Lillenthal and Dr. Riley. We have found that the dissociation curve is not different from the normal curve; in other words, it does follow the curve which is considered as a standard curve. This indicates that hemoglobin is not different from normal hemoglobin.

*Dr. Arnold Rich:* May I ask Dr. Bing whether he found any cases in his studies that would indicate that the collateral circulation is equal to or greater than the pulmonary flow?

*Dr. Bing:* There are such cases in which the collateral circulation exceeds the volume of flow through the pulmonary artery. This is especially true in cases of complete pulmonary atresia. Drs. Blalock and Duncan have confirmed this.

*Conversion of Normal into Malignant Cells in Vitro.* DR. GEORGE O. GEY  
(Department of Surgery).

This study is concerned with a series of permanent alterations occurring in continuous cultures of normal rat mesenchyme cells and leading to the production of malignant cells. The strains studied include normal, altered normal, and malignant cell strains, all of autologous origin, which have been cultivated in roller tubes for eight and one-half years. These strains make it possible to make direct comparisons between a normal and a malignant strain derived therefrom. The data to date suggest that factors contributed by the culture medium, which is totally heterologous, are important and may play a role in the alterations observed. To date, no known extraneous carcinogenic agents have been found which might have brought about these conversions which occurred in stocks of normal cell strains. In this presentation some of the differences between the normal and the malignant autologous strains were discussed. Certain differences in morphology and activity were illustrated with lantern slides and phase-contrast motion pictures.

#### DISCUSSION

*Dr. Wilton Earle* (National Cancer Institute): I didn't intend to come here to talk, but just to hear this interesting work reported by Dr. Gey. I have been following Dr. Gey's work for quite a number of years, and meanwhile our own work has been carried on along very similar lines since in our work we have been treating normal mouse fibroblasts with the carcinogen 20-methylcholanthrene. In a num-

ber of ways our results parallel Dr. Gey's. In our studies we did get a great change in our treated cultures and a slight change in our untreated control cultures. The change in the control cultures was a slight one and the cells were just perceptibly altered morphologically from the normal, yet that strain of cells has given a greater percentage of sarcomas on injection than any of the cell strains deliberately treated with the carcinogen; one group of cultures of this strain gave us 100% sarcomas on injection. Sections of these sarcomas showed very slight anaplasia; metastases have not occurred, and while there was infiltration there was little cachexia. With cultures substantially more altered by deliberate addition of the carcinogen for a short interval of days, we obtained a smaller percentage of sarcomas produced on injection of the treated cells, but in sections of these sarcomas there was greater anaplasia and metastases did occur.

We do not yet know what agent or agency caused the limited degree of neoplastic change in the control cultures, but I think it very likely they received an accidental trace contamination with the same carcinogen. Leaving the behavior of these control cultures out of consideration, however, the results from our cultures indicate that the greater the exposure time of the cells to a constant concentration of carcinogen, the greater the change in the cells. Further, I think we can say that in our cultures at least, this change for any one culture seems reasonably uniform throughout the culture. In Dr. Gey's cultures it was most interesting that he found his changes occurring in small groups of cells; in our studies the cultures appeared to change as a whole.

We are just completing the tabulation of our data and I think we can now say that of the six strains of neoplastic cells we have originated in vitro, all from one parent strain of normal mouse fibroblasts, it now appears that three out of the six have preserved their altered morphology without further change for over four years since their removal from the carcinogen. Three strains do show some continued change, but certain recognizable characteristics peculiar to each other have persisted unchanged. This apparent continued change since removal from carcinogen may, however, be due in whole or in part to secondary cell selection of more hardy cells in propagation of the cultures. In the remaining three strains, however, there has been no further recognizable change in their morphology for over 4 years in culture. In these three strains, if we allow, generously, 5 days for the average intermitotic interval of their cells, it then appears that the cells of these strains have been able to stabilize their respective altered morphologies so that they have been preserved through more than 300 consecutive generations of cell mitosis since removal of the cells from carcinogen.



# SYMPOSIUM ON VAGOTOMY FOR PEPTIC ULCER

## I. EXPERIMENTAL OBSERVATIONS\*

HENRY N. HARKINS, DONALD H. HOOKER, T. CRANDALL ALFORD, JR.,  
JOHN CALLANDER, STUART R. ELLIOTT, II, WALTER KEARNS, JR.,  
JAMES MITCHENER, AND DENTON A. COOLEY

*From the Department of Surgery, Johns Hopkins University and Hospital, and the Surgical Hunterian Laboratory*

With the current interest in the clinical use of vagotomy for the treatment of peptic ulcer, it is felt that any experimental observations bearing on this subject are pertinent. This is particularly true since many of the older experimental studies involved the use of vagotomy done at other points along the course of the vagus nerves than does the modern clinical procedure.

The present studies can be divided into six groups, three of which directly involve the use of vagotomy and two of which explore the possibility of the use of an anti-histamine drug, benadryl, in the control of experimental ulcer. These six groups of experiments are as follows:

1. An attempt to produce histamine ulcers in the rat.
2. The use of aqueous benadryl solution in an attempt to prevent histamine-in-beeswax ulcers in the guinea pig.
3. The use of benadryl-in-beeswax in an attempt to prevent histamine-in-beeswax ulcers in the guinea pig.
4. The use of vagotomy in an attempt to prevent histamine-in-beeswax ulcers in the guinea pig.
5. The use of vagotomy in preventing pyloric ligation-induced ulcers in the Shay rat.
6. The influence of vagotomy on the development of jejunal ulcers in the Mann-Williamson dog.

These experiments will now be considered individually:

1. *An attempt to produce histamine ulcers in the rat.* In a series of four albino rats using larger doses (20 to 80 mgm. of histamine base daily) of histamine-in-beeswax than were used in guinea pigs (see

\* Paper given as part of a Symposium on Vagotomy For Peptic Ulcer at the April 14, 1947 meeting of the Johns Hopkins Medical Society.

Section 2), no ulcers were produced after from one to 24 daily injections. These results are compatible with the well known relative immunity of rats towards histamine. Possibly, if the rats had been starved, as they were not in these experiments, ulcers might have formed.

2. *The use of aqueous benadryl solution in an attempt to prevent histamine-in-beeswax ulcers in the guinea pig.* Using the method of Varco, Code, Walpole, and Wangenstein (1941), guinea pigs were given daily subcutaneous injections of histamine-in-beeswax, the usual dose being 5 mg. of histamine base. In a series of 29 guinea pigs, twenty, or 69 per cent, developed peptic ulcers. As shown in Table I, five of seven guinea pigs given similar doses of histamine-in-beeswax de-

TABLE I

*Ulcer Formation in Guinea Pigs Following Subcutaneous Histamine-in-Beeswax Injections: Fifty-Three Successful Experiments*

PROCEDURE	ULCER OR EROSION FORMATION	PER CENT ULCER
		%
Control histamine-in-beeswax (H.I.B.)	20 of 29 guinea pigs	69
H.I.B. plus benadryl-in-saline per os	5 of 7 guinea pigs	72
H.I.B. plus benadryl-in-saline subcutaneously	8 of 8 guinea pigs	100
H.I.B. plus benadryl-in-beeswax subcutaneously	4 of 4 guinea pigs	100
H.I.B. plus vagotomy	5 of 5 guinea pigs	100
Total or average. . . . .	42 of 53 guinea pigs	79

veloped ulcer, or 72 per cent, despite the administration of aqueous benadryl solution<sup>1</sup> by mouth (a daily dose of 2 to 4 ml. of benadryl solution; 1 ml.  $\equiv$  10 mgm.).

In additional experiments all of eight guinea pigs (100 per cent) given histamine-in-beeswax plus daily doses of 2 to 3 ml. of benadryl subcutaneously developed peptic ulcers within 2 to 3 days. Seven pigs died with perforated ulcer(s) and the remaining animal died with an unperforated ulcer.

3. *The use of benadryl-in-beeswax in an attempt to prevent histamine-in-beeswax ulcers in the guinea pig.* All four of a series of guinea pigs

<sup>1</sup> Kindly furnished by Parke, Davis and Co.

(100 per cent) treated developed ulcers and died within 1 to 5 days. The daily dosage of benadryl used was 0.1 to 0.4 ml. benadryl-in-beeswax subcutaneously (1.0 gm. benadryl + 1.0 ml. beeswax + 3.5 ml. mineral oil: mix. 1 ml. mixture = 250 mgm. benadryl). Two other guinea pigs died from histamine reactions immediately after injection.

4. *The use of vagotomy in an attempt to prevent histamine-in-beeswax ulcers in the guinea pig.* Infradiaphragmatic section of the vagus nerves was done in a series of guinea pigs under open inhalation ether anesthesia. The immediate mortality of the procedure was high because of the difficulty in adequately exposing the vagi without opening the esophageal hiatus and producing pneumothorax. More latterly this difficulty was somewhat overcome by the use of moist cotton packs around the esophagus. Parenthetically it might be mentioned that this difficulty was not at all a problem in vagotomy on rats (see Section 5, below).

After recovery from the operation, the animals were given the usual daily dose of histamine-in-beeswax and all of five (100 per cent) developed ulcers. Three other guinea pigs developed ulcer, but there was evidence that the vagotomy may not have been complete.

In the entire series of 95 guinea pigs shown in Table II, only 53 were included in the above discussion. The remaining 42 were eliminated because of acute toxicity death (usually from histamine)—25, incomplete vagotomy—3, and operative mortality—14.

5. *The use of vagotomy in preventing pyloric ligation-induced ulcers in the Shay rat.* It has been shown by Shay, Komerov, Fels, and Meranze (1945) that starved rats regularly develop multiple hemorrhagic ulcerations of the gastric rumen, as well as occasional ulcers of the gastric fundus, within 15 hours following pyloric ligation. In a preliminary report, we showed that vagotomy abolishes this tendency to ulcer formation.

*Technic:* Littermate rats weighing about 200 gms were starved for 72 hours, but were allowed water. Operation was performed under inhalation ether anesthesia plus intraperitoneal pentothal (0.6 cc. 1 per cent solution/200 gm. rat). The stomach was exposed through a short vertical epigastric incision using aseptic precautions and the pylorus was ligated in all animals. In the vagotomized half of the series the vagus nerves were completely and tightly ligated



just below the diaphragm. This ligation was done in a manner similar to that used for carotid sinus nerve ligation in Heyman's laboratory, where the ligature surrounded everything but the carotids. In our experiments the ligature surrounded everything in the region except the esophagus. The animals were then replaced in cages without food or water after resuturing the abdominal wound.

*Results:* In the series of rats killed at the end of 24 hours, twenty-two control rats presented an average of 22 ulcers of the rumen per rat. In addition, eight rats had ulcers of the fundus and two had

TABLE II

*Detailed Analysis of All Ninety-Five Guinea Pigs Used in Histamine-in-Beeswax Experiments*

FINDING	H.I.B.	H.I.B. PLUS BENADRYL- IN-SALINE PER OS	H.I.B. PLUS BENADRYL- IN-SALINE SUBCUTANE- OUSLY	H.I.B. PLUS BENADRYL- IN-BEESWAX SUB-CUTANE- OUSLY	H.I.B. PLUS VAGOTOMY	TOTAL
No ulcer . . . . .	9	2	0	0	0	11
Erosion . . . . .	2	2	0	0	0	4
Ulcer . . . . .	18	3	8	4	5(8)†	41
Acute toxicity death	10*	1	0	3‡	11	25
Operative mortality ..					14	14
Total. . . . .	39	8	8	7	33	95

\* These animals were not autopsied and some may have had ulcers when death followed other than the initial injection.

† The larger figure includes 3 animals in which the completeness of the vagotomy is doubtful.

‡ One of these three animals was not autopsied.

perforated ulcers. In the series of 20 vagotomized rats, no ulcers were present.

One possible factor in the protective action of vagotomy may be the decrease in the hydrochloric acid content of the stomach contents found at autopsy. In the control series the free acid averaged 34 units and the total acid 94 units, while in the vagotomized group the values were 8 and 66 respectively. The decrease in free acid cannot, however, be the entire explanation of the beneficial effect of vagotomy because in individual experiments 7 vagotomized rats, all without ul-

cer, had a higher gastric acidity than 10 companion control rats, all with ulcer.

Another possible factor in the protective action of vagotomy may be the decrease in gastric volume of the stomach found at autopsy. In the control series, the fluid content of the stomach averaged 15

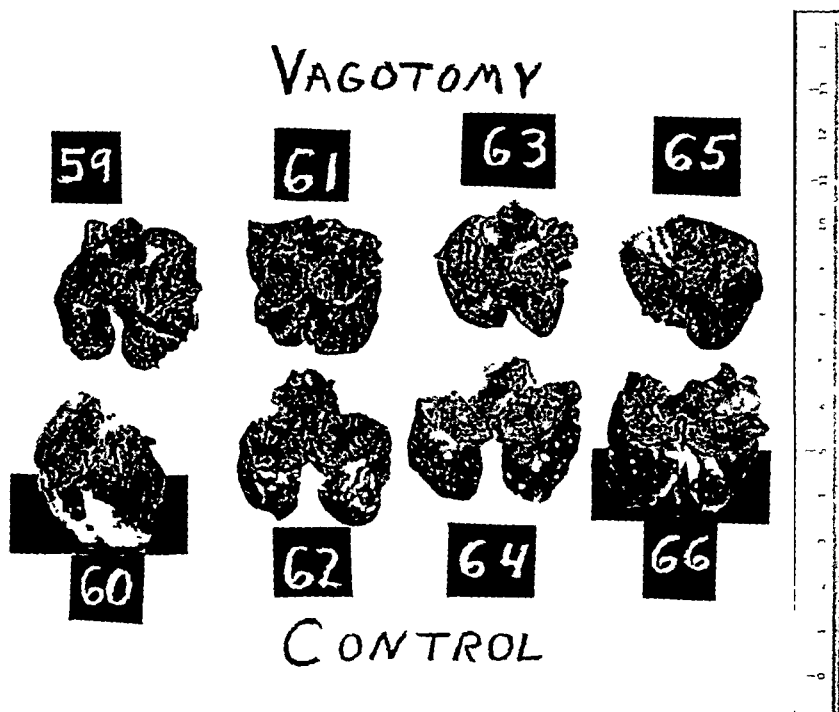


FIG. 1. THE EFFECT OF VAGOTOMY IN PREVENTING PYLORIC LIGATION-INDUCED ULCERS IN THE SHAY RAT

In the upper row, four rat stomachs show no ulcers after the combined operation. In the control stomachs in the lower row, where only pyloric ligation and no vagotomy was done, numerous ulcers of the rumen are present.

ml. while in the vagotomized group it was only 7 ml. This factor alone cannot be the entire explanation of the beneficial effect of vagotomy because in the control series five rats with a gastric volume of 8 ml. or less presented 126 ulcers while in the vagotomized series, seven rats with a gastric volume of 8 ml. or more had no ulcers.

It is of interest that the gastric contents were almost invariably frothy in the vagotomized series whereas in the control animals there was never froth. The factor of swallowed saliva was considered and a series of esophageal ligations was done in addition to the pyloric ligations and in some instances to vagotomy as well. When esophageal ligation was done at the cardia (plus pyloric ligation) only 1 of 7

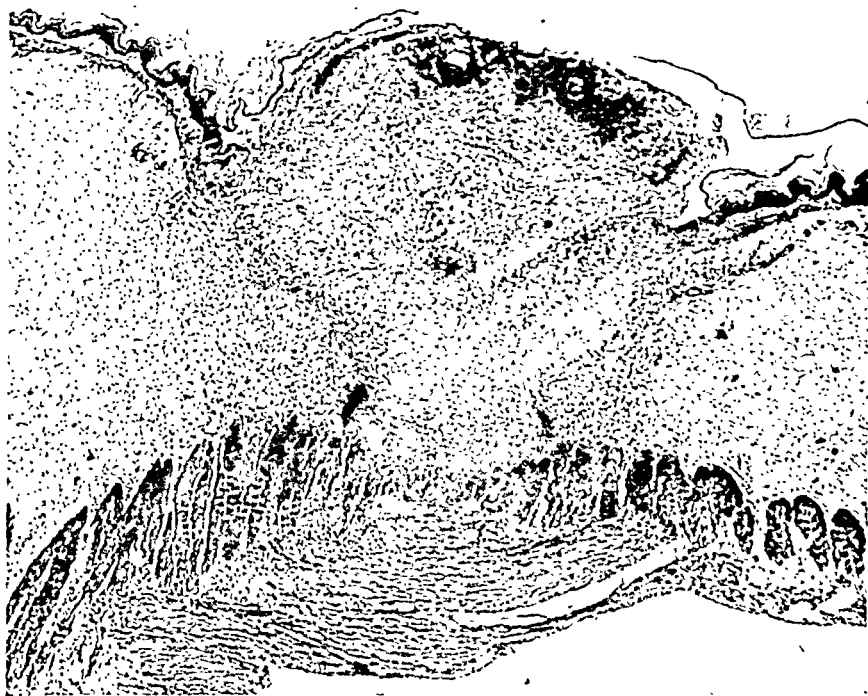


FIG. 2. ULCERATION AND EDEMA OF WALL OF GASTRIC RUMEN 24 HOURS AFTER PYLORIC LIGATION. ( $\times 37\frac{1}{2}$ )

rats developed ulcer. The maximum gastric volume was only 1.5 ml.; the free acid 56–80, average 72 units; and the total acid 144–184, average 163. Five other rats were also vagotomized and none of these developed ulcers.

It is soon noted, however, that esophageal ligation at the cardia, even without supplementary vagotomy, invariably produced necrosis of the esophagus up to the level of the tracheal bifurcation, evidently

from interference with the blood supply of the lower esophageal segment. To get around this difficulty esophageal ligations were performed in the neck. One of 6 rats (esophageal ligation in the neck plus pyloric ligation) developed ulcers. The maximum gastric volume was only 6.5 ml.; the free acid 16-80, average 54; and the total acid

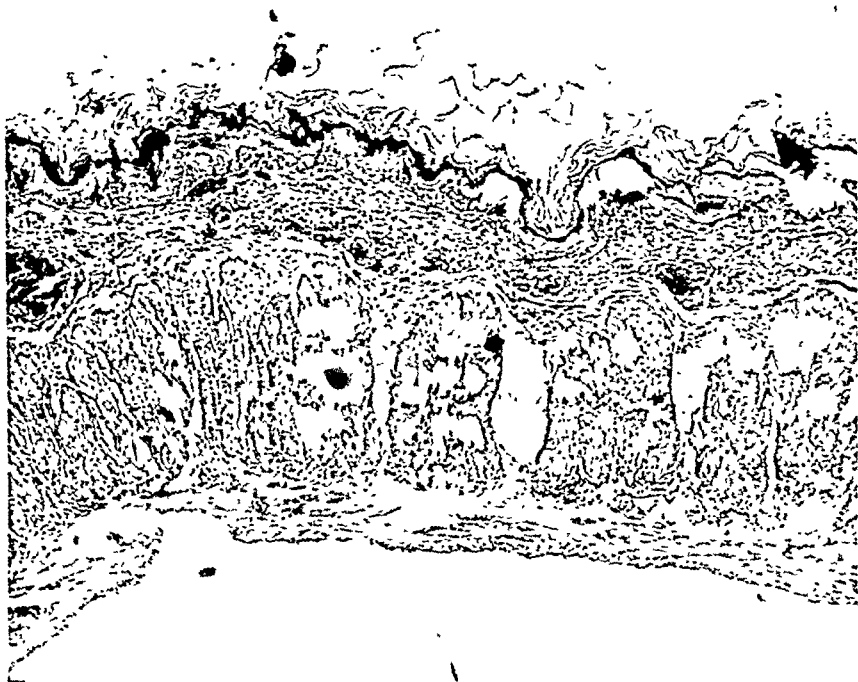


FIG. 3. LACK OF ULCERATION OR EDEMA OF WALL OF GASTRIC RUMEN 24 HOURS AFTER PYLORIC LIGATION IN RAT IN WHICH VAGOTOMY WAS ALSO DONE. ( $\times 62\frac{1}{2}$ )

110-204, average 146. Four other rats were in addition vagotomized with no ulcers resulting.

As has been already reported (1947), vagotomy not only prevents ulcer formation in rats with pyloric ligation, but in survival experiments prolongs the life of the animals. In a series of 10 control rats with pyloric ligation, the average duration of life was 49 hours, whereas the series of 10 other rats with additional vagotomy lived an average

of 106 hours. Both groups of animals received intraperitoneal glucose-saline injections. Furthermore, protein analysis of the gastric fluid removed at death indicated such a small amount of protein as to rule out death from acute protein loss.

6. *The influence of vagotomy on the development of jejunal ulcers in the Mann-Williamson dog.*<sup>2</sup> An experiment simulating the problem faced by the clinical surgeon and at the same time testing the effect of vagotomy on the development of jejunal ulcer was performed by Beaver and Mann (1931). These authors reported experiments on 6 dogs and found that jejunal ulcers occurred in 100 per cent of their three control animals whereas 67 per cent of the three dogs which had

TABLE III  
*Results of Vagotomy in 22 Mann-Williamson Dogs*

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*13 Controls*

- 11 developed ulcer (85%)
- Survival 29-161 days (average = 64 days)
- 2 died with no ulcer
- Survival 55-145 days (average = 100 days)

*9 with Vagotomy*

- 1 developed ulcer (11%)
- Survival 41 days
- 8 had no ulcer
- 6 died Survival 28-197 days (average = 118 days)
- 2 living Survival 263-487 days (average = 375 days)

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8 total Survival 28-487 days (average = 182 days).

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been subjected to both the Mann-Williamson operation (1923) and the vagotomy developed ulcers. It is the purpose of this paper to report results to date on a series of 22 dogs prepared similarly to those of Beaver and Mann.

*Technique:* The Mann-Williamson operation (Figure 4) was done according to the principles laid down by these authors. Using aseptic technique, the abdomen was opened through a right rectus incision. The pylorus was divided and the duodenal stump inverted. Approximately 15-20 cm. below the ligament of Treitz, the jejunum was transected, the distal end then being anastomosed to the pyloric end of

<sup>2</sup> The authors acknowledge the assistance of Mr. Thomas Satterfield in this group of experiments.

the stomach and the proximal end being anastomosed to the ileum. End-to-end anastomoses were done whenever possible at the pylorus and end-to-side anastomoses at the lower junction. Continuous fine chromic catgut was used for the mucosal layer and one layer of interrupted or continuous silk for the serosal layer.

The vagotomy was done through the 6th interspace on the left using intratracheal ether anesthesia and a pressure machine. Segments of vagus nerve 1-2 cm. long were removed immediately above the diaphragm and the stumps ligated with silk. An anterior trunk, several small lateral branches and a large posterior trunk were usually demon-

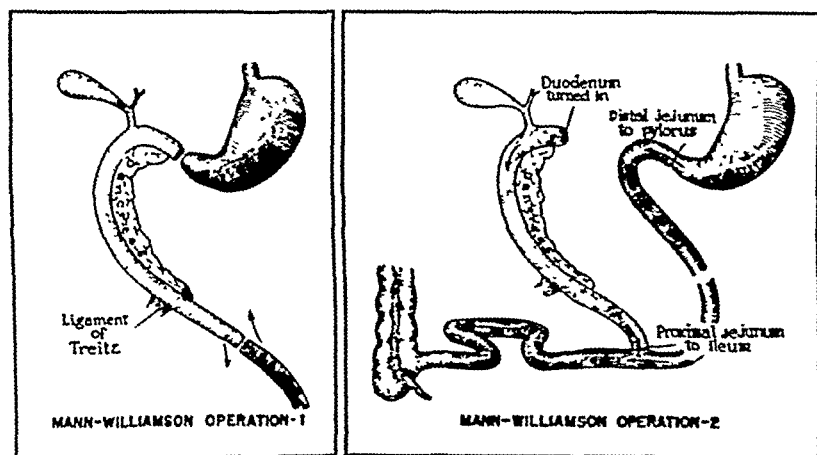


FIG. 4. DIAGRAM OF MANN-WILLIAMSON PROCEDURE

strable. The posterior trunk was made up of a number of small lateral and posterior branches which decussated several centimeters above the diaphragm. The pleural defects, subcutaneous tissues and skin were closed with fine silk. The separation between the ribs was closed by approximating the ribs with heavy braided silk after inflating the lungs with care for it was at this juncture that anesthetic deaths are apt to occur.

*Discussion of data:* To eliminate the immediate effects of the operation on the evaluation of the incidence of jejunal ulcer, the dogs reported are limited to those which survived at least 28 days following the Mann-Williamson procedure. It was observed that after the

dogs had recovered from the operation (i.e. several weeks) their general condition improved for a few more weeks. Then they began to lose weight, vigor, and appetite and in this particular series, on an average of 64 days after operation they succumbed to a perforated jejunal ulcer. An effort was made to do the vagotomy as early after the Mann-Williamson operation as possible to eliminate the possibility of error due to the selection of animals which would not have developed ulcer anyway. However, due to distemper and other uncontrollable factors the average time between the Mann-Williamson operation and vagotomy was 51 days. It is interesting that at the time of vagotomy the dogs had already lost, on an average, 16 per cent of their body

TABLE IV  
*Correlation of Length of Survival and Loss of Body Weight in Mann-Williamson Dogs With and Without Vagotomy*

CONTROLS	AVERAGE DAYS SURVIVAL	AVERAGE LOSS BODY WEIGHT
11 with ulcer	64	42%
2 without ulcer	100	33%
WITH VAGOTOMY		
1 with ulcer	41	more than 35%
6 without ulcer	118	40%
2 probably without ulcer	375	2% (gain)

weight. Another series is now being done with the vagotomy first to eliminate this possible discrepancy.

Table I shows the control series of 13 Mann-Williamson dogs of which 11 or 85 per cent were proved by autopsy to have ulcer and two not to have ulcer. Of the 11 dogs with ulcer 8 died with a perforation, one was sacrificed, and two died of inanition.

The dogs with ulcer died on an average of 64 days following operation whereas the two without ulcer survived an average of 100 days; one dying 55 days after operation from distemper and the other having been sacrificed 145 days after operation because of severe mange.

The second series is represented by nine dogs which had been subjected to the Mann-Williamson operation and then vagotomy.<sup>3</sup> Six

<sup>3</sup> An additional dog, which had vagotomy performed 95 days before the Mann-Williamson operation and which died 56 days after the latter operation, had no

of these dogs, proven by autopsy to have no ulcer, lived an average of 118 days. Two others are living and well and are therefore presumed not to have an ulcer, having survived 263 and 487 days respectively. The one dog in this series with ulcer died 41 days after the Mann-Williamson operation.

Table II is an effort to correlate the weight loss with the survival time. We find that in the control series the dogs with ulcers lost 9 per cent more weight and their life span was on an average 36 days shorter than those dogs without ulcers. The Mann-Williamson dogs with vagotomy which lost 40 per cent body weight did not have ulcer, and although they lost as much weight as the control dogs with ulcer, the vagotomized dogs lived on an average 54 days longer.

In an attempt to analyze the mortality figures of the thirty-five deaths in one series, we find that eighteen dogs died from peritonitis due to leaks at the anastomoses, one died from evisceration, seven died from infection at the operative site, two died from distemper and seven died from unknown causes. In another series of twenty-eight dogs, nine died from leaking anastomoses, one from infection, one from evisceration, three from gastric retention following the vagotomy, six from anesthesia, four from distemper and four from causes unknown. Roughly one dog out of every four operated on could be used for the summary in Table I.

#### DISCUSSION

The absence of protective action of benadryl against histamine-provoked ulcers in guinea pigs agrees with the results of Friesen, Baronofsky, and Wangenstein (1946) who obtained similar results in dogs. This result may be partially explained by an insufficient dosage of benadryl, but is otherwise difficult to understand entirely. On the other hand, the absence of protective action of vagotomy against histamine provoked ulcers can be explained by the peripheral

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ulcer at autopsy. Inclusion of this dog in the series gives an ulcer incidence of 10 per cent, an average survival of vagotomy dogs that died of 109 days, an average survival of all vagotomy dogs without ulcer of 155 days, an average body weight loss of 40 per cent and an average interval between the two operations of 56 days.



action of histamine. The protective action of vagotomy against pyloric ligation-induced ulcers is of extreme interest. Peptic ulcer occurred in 85 per cent of the dogs on which the Mann-Williamson operation had been done and in 11 per cent of the dogs on which both the Mann-Williamson operation and vagotomy had been done, but because of the rather long interval between the Mann-Williamson and the vagotomy (i.e. an average of 51 days) the possibility of error due to selection of cases must be considered and the above results viewed with caution.

### CONCLUSIONS

1. Benadryl (per os or subcutaneously; in aqueous or in beeswax-in-oil solution) does not prevent histamine-provoked peptic ulcers in guinea pigs within the limits of dosage and conditions of the experiments reported.
2. Vagotomy does not prevent histamine-provoked ulcers in guinea pigs within the limits of dosage of the drug as used.
3. Vagotomy prevents the development within 24 hours of ligation-induced ulcers in rats and lengthens the life of such animals.
4. Vagotomy lessens the acidity and volume of the accumulated gastric fluid following pyloric ligation in rats, but neither of these factors alone explains the beneficial effect of this procedure.
5. Esophageal ligation, with or without vagotomy, reduces the incidence of pyloric ligation-induced ulcers in rats, but does not entirely prevent them.
6. Vagotomy performed an average of 51 days after Mann-Williamson operations in dogs reduced the incidence of peptic ulceration following this operation from a control level of 85 per cent to 11 per cent.

### BIBLIOGRAPHY

1. BEAVER, M. G., AND MANN, F. C.: The Production of Peptic Ulcer After Section of the Gastric Nerve. *Ann. Surg.* 94: 1116-1118 (Dec.) 1931.
2. FRIESEN, S. R.; BARONOFKY, I. D., AND WANGENSTEEN, O. H.: Benadryl Fails to Protect Against the Histamine Provoked Ulcer. *Proc. Soc. Exp. Biol. & Med.* 63: 23-25 (Oct.) 1946.
3. HARKINS, H. N.: The Prevention of Pyloric Ligation-Induced Ulcers of the Gastric Rumen of Rats by Transabdominal Vagotomy: A Preliminary Report. *Bull. Johns Hopkins Hosp.* 80: 174-176 (March) 1947.

4. MANN, F. C., AND WILLIAMSON, C. S.: The Experimental Production of Peptic Ulcer. *Ann. Surg.* 77: 409-422 (April) 1923.
5. SHAY, H.; KOMEROV, S. A.; FELS, S. S.; MERANZE, D.; GRUENSTEIN, M., AND SIPLET, H.: A Simple Method For the Uniform Production of Gastric Ulceration in the Rat. *Gastroent.* 5: 43-61 (July) 1945.
6. VARCO, R. L.; CODE, C. F.; WALPOLE, S. H., AND WANGENSTEEN, O. H.: Duodenal Ulcer Formation in the Dog by Intramuscular Injections of a Histamine Beeswax Mixture. *Am. J. Physiol.* 133: 475-476 (June) 1941.

# SYMPOSIUM ON VAGOTOMY FOR PEPTIC ULCER

## II. EARLY SURGICAL RESULTS IN FORTY-THREE CASES\*

THOMAS N. P. JOHNS, AND WILLIAM E. GROSE

*From the Department of Surgery of the Johns Hopkins University and the Johns Hopkins Hospital*

Vagotomy for peptic ulcer has been performed on 43 patients at the Johns Hopkins Hospital. The purpose of this tentative report is to present the early results in these cases, along with certain post-operative observations.

The first operation here was performed just two years ago. It is obvious, therefore, that the post-operative intervals in these studies are not long enough to permit any valid interpretations. Furthermore, this group of patients lacks the clinical and pathologic uniformity requisite to the evaluation of any operative procedure. Vagotomy, either alone or in combination with some other operation for ulcer, has been performed for all the common complications of peptic ulcer. No new or decisive findings are recorded, but the fact that this operation with several modifications has produced interesting and inconstant changes in a varied group of cases would seem to justify an early report.

### CLINICAL MATERIAL

In this series 4 different operative procedures have been used. In order that each of these procedures may be considered separately, the patients are arbitrarily divided into 4 corresponding groups (Table 1). Transthoracic vagotomy with division of the greater splanchnic nerve on one side was performed on 3 patients (Group I) including the first cases in the series. The rationale for this procedure, which was first used here by Dr. William P. Longmire, Jr., was based on the experimental work of Pereira (12), who found a marked vasodilatation of the smaller blood vessels of the pylorus with increase in collateral blood flow to this region following division of the right greater

\* Paper given as part of a Symposium on Vagotomy For Peptic Ulcer at the April 14, 1947 meeting of the Johns Hopkins Medical Society.

splanchnic nerve in dogs. Vagotomy alone (Group II) was done in 16 cases, 5 by the abdominal approach and 11 transthoracically. This group was begun by Dr. Harris B. Schumacker, Jr. in April, 1946. Groups III and IV include patients who have been subjected to some plastic or ablative procedure on the stomach along with vagotomy. Artificial stomata were created in 10 patients (Group III): 8 gastroenterostomies and 2 Finney pyloroplasties. Subtotal gastric resection was performed in 14 cases (Group IV). The amount of

TABLE 1  
*Type of Operation*

GROUP	PROCEDURE	CASES
I	Vagotomy & Splanchnicotomy	3
II	Vagotomy alone	16
	Transthoracic	11
	Abdominal	5
III	Vagotomy & Stoma	10
	Gastroenterostomy	8
	Pyloroplasty	2
IV	Vagotomy & Resection	14
	Polya	7
	Hofmeister	7
Total		43

stomach removed was about 60% on the average. The gastrojejunal anastomoses were of the Polya type in 7 patients and of the Hofmeister type in 7.

The technique of vagotomy was similar to that described by Dragstedt (2, 3, 4, 5, 6), Alvarez (1), and others (15). When the transthoracic approach was chosen, the chest was entered posterolaterally through the bed of the resected left 8th rib or 8th interspace and the supradiaphragmatic esophagus freed of all detectable vagal fibers over a considerable area. The nerves were then divided and the distal ends sutured into the mediastinal pleura usually after resection of nerve segments 2 to 3 inches in length. In the abdominal approach an upper midline incision was used. (the left lobe of the liver reflected

after division of the left triangular ligament) and 2 or 3 inches of esophagus mobilized from above by blunt dissection. The esophagus was then bared of the two vagal trunks, a careful effort being made to include all the ramifications over an area from just above the cardia to the highest proximal point within reach. The free segments of nerve were then resected and the proximal ends sutured into the diaphragmatic peritoneum. In a few cases the ends were allowed to retract far into the mediastinum, after which the esophageal hiatus was carefully peritonealized.

In the entire group of 43, there were 32 duodenal lesions, 9 gastric, and 2 marginal. Of the 9 gastric ulcers, 5 were in the resected group, 1 other was biopsied, and the remaining 3 visualized were grossly benign.

#### INDICATIONS

This series embraces, as noted above, most of the complications and complexities of the peptic ulcer problem. The primary indications for surgery in the patients in each group are shown in Table 2. In several cases, of course, additional circumstances, for example, the presence of a gastric lesion, made operation advisable. In over half the cases, ulcer pain constituted the chief indication for surgery. For the most part, this was the so-called uncomplicated pain, either intractable, recurrent and severe, or pain not controlled by the best medical regimen environmentally possible. In 7 of the 8 patients in whom massive hemorrhage or pyloric obstruction were indications, either gastroenterostomy or resection was performed along with vagotomy. There were 9 patients admitted to the hospital for repair of perforations. At intervals of 10 to 14 days following closure of perforation, these patients were submitted to transthoracic vagotomy with the hope that healing of the ulcer would be facilitated and as a safeguard against future difficulty. It is true that while all of these patients had been having ulcer symptoms for variable periods preceding perforation, none would have been considered in need of immediate ulcer surgery other than repair of perforation and probably a few of these could have been carried satisfactorily on medical regimen. One other patient underwent vagotomy at the time of repair of perforation. This was a unique case of ruptured Cushing ulcer occurring a month following removal of a brain tumor in a 6-year-old boy. This

patient lived 2 weeks after vagotomy with apparent resolution of abdominal difficulties and died of meningitis and dural sinus throm-

TABLE 2  
*Primary Indications for Vagotomy*

GROUP	NO. PTS.	PAIN	PERFORATION	OBSTRUCTION	HEMORRHAGE
I (With Splanchnicotomy)	3	2	1	0	0
II (Vagotomy alone)	16	8	7	0	1
III (With Stoma)	10	7	1	1	1
IV (With Resection)	14	8	1	2	3
Total.....	43	25	10	3	5

TABLE 3  
*Location of Ulcer*

GROUP	NUMBER OF PATIENTS	LOCATION OF ULCER		
		Gastric	Duodenal	Marginal
I (With Splanchnicotomy)	3	1	1	1
II (Vagotomy Alone)	16	2	13	1
III (With Stoma)	10	1	9	0
IV (With Resection)	14	5	9	0
Total.....	43	9	32	2

bosis. This is probably the youngest patient on record upon whom vagotomy has been done for ulcer and the only one performed for Cushing ulcer.

## RESULTS

*1. Follow-up*

With the exception of the 2 patients previously cited in Group I, whom Dr. Longmire operated upon 2 years ago, the patients longest followed in the series are now only 12 months post-operative, the average overall period of study being 6 months. Of the 43 patients on whom vagotomy was performed, 35 have been followed in the Gastro-Intestinal Clinic under the surveillance of Drs. Paulson and Gladsden. Also, detailed questionnaires have been mailed to all patients. In this way, 41 patients have been followed. According to current status, 34 of the 41 have done satisfactorily. In 7 patients the result has been unsatisfactory. The 2 patients not followed were in the group undergoing resection in addition to vagotomy and were both doing well at the time of discharge.

*2. Symptom Changes*

*A. Pain.* Ulcer pain has disappeared promptly and dramatically in all patients following operation. This effect has been particularly convincing in patients who were experiencing severe pain immediately beforehand. It was noted that the disappearance of pain has enabled many of these people to surmount the usual post-operative difficulties with uncommon ease and speed. In the unsatisfactory group, 3 patients experienced recrudescence of ulcer pain along with reactivation of their ulcers after periods of 5, 10, and 11 months. Of these, 2 underwent right major splanchnicotomy at the time of vagotomy and subsequently showed evidence that all the vagal fibers had not been divided. There are 3 other patients in the unsatisfactory group who have complained of abdominal discomfort, but in these cases the pain has been that of gastric dilatation and not ulcer pain. Excepting 2 patients in Group IV who have noted occasional mild twinges of abdominal pain, none of the patients followed in Groups III and IV have experienced pain.

*B. Vomiting.* Despite the fact that continuous gastric suction was used routinely for the first 2 or 3 post-operative days, 9 patients experienced considerable vomiting while still on the surgical wards. Such episodes were related to the gastric atony and dilatation which

characteristically followed vagotomy and not to preoperative symptomatology. This early vomiting is a post-operative complication which can be controlled and probably prevented by adequately prolonged continuous removal of gastric contents followed by carefully graduated diet. Only 2 patients in the satisfactory group have been troubled with vomiting since discharge. One of these had to be readmitted 3 weeks after operation for gastric decompression. In both, the difficulty disappeared within a month after operation.

Of the 6 unsatisfactory cases with troublesome post-vagotomy vomiting, 4 have come to reoperation. Active peptic ulceration was present in 3 of these cases, 2 being the incompletely vagotomized patients in Group I and the third, a man resected 5 months after transthoracic vagotomy, whose denervation was complete according to the insulin test. The fourth patient had an atonic stomach, but the ulcer had healed. No sign of pyloric obstruction was present in any of the 4 cases. In fact, in 3 patients having clinical and roentgenological signs compatible with a diagnosis of pyloric stenosis, the pyloric rings were palpated carefully at reoperation and were found to be free and patulous, the stomachs atonic and dilated, and, in 2 cases, the proximal portions of the duodenum somewhat larger than normal. It should be said that one of these patients, despite the absence of organic obstruction, had been shown to have slight impairment of gastric motility and emptying even before vagotomy, although vomiting was never present until afterward. Resections were performed on the 3 patients with active ulcers and a gastroenterostomy on the patient with gastric dilatation. All are now doing well. In the unsatisfactory group, 2 other patients, now 10 and 4 months after vagotomy, are having troublesome vomiting.

*C. Hemorrhage.* Before operation, 16 patients gave a history of significant bleeding, massive hemorrhage being the chief indication for surgery in 5 of these. There has been no proved instance of hemorrhage in any patient following vagotomy, although 2 patients gave a history of having had small tarry stools 7 and 8 months post-operatively.

*D. Fullness after Eating.* Transient post-prandial distress has been common as a post-operative complaint present to varying degrees in 29 patients. This symptom usually disappeared or abated



greatly within the first month, though at the present time 22 patients state that they are aware of occasional, mild fullness, especially after overeating. Except for patients in the unsatisfactory group, in 6 of whom severe post-prandial fullness was added to their other troubles, this symptom has not been a real problem. Dietary limitations have been advised in the group with resections and in other patients only as needed to control symptoms of fullness. Under this policy, there are now 22 patients eating everything and 11 patients on frequent-feeding regimens, the latter group including those with resections or

TABLE 4  
*Changes in Bowel Habits and Weight following Vagotomy*

GROUP	NO. OF PATIENTS	INCREASED DEFECCATORY RATE	DIARRHEA	AVERAGE WEIGHT GAIN
I (With Splanchnicotomy)	3	0	0	pounds 0
II (Vagotomy Alone)	16	16	4	19
III (With stoma)	10	9	2	16
IV With Resection	14	8	2	13
Total.....	43	33	8	15

others in the early post-operative period. The incidence of dietary limitation is greater (64%) in the resection group than in the satisfactory cases in the other groups (17%). This rather high figure includes all patients dieting and does not represent the true incidence of digestive cripples since feeding restrictions have been advised in some cases in the absence of symptomatic indications.

*E. Bowel Habits.* 33 patients experienced a post-vagotomy increase in their daily defecatory rate. Since 26 patients had constipation before vagotomy, this abrupt change, in most instances, has proved salutary. However, 8 patients were bothered with diar-

rhea for post-operative periods of 4 to 12 weeks. This consisted usually of 5 to 10 soft or semi-solid stools a day, typically as diarrhea after eating, and has been controlled satisfactorily by the use of pavetrine and phenobarbital. At present, one patient who had vagotomy with gastroenterostomy 3 months ago is having severe diarrhea. Despite diarrhea, weight gain has been uniformly striking.

*F. Attitude.* Particularly striking has been the enthusiasm of the patients themselves about the operation. The absence of pain seems to be the key to this fact, since even those having diarrhea and post-prandial fullness tend to minimize these complaints in expostulating over their freedom from ulcer pain. All patients state that they would go through the operation again, knowing what they now know, with the exception of one man now doing well, whose objection was the many "tubes and needles" he had to cope with in the hospital.

### 3. *Secretory Changes*

The changes in gastric secretory function which have followed vagotomy are presented in an accompanying article by Drs. Paulson and Gladsden in their studies on patients followed in the Gastro-Intestinal Clinic. All groups have shown a considerable reduction in the volume of 12-hour resting secretion and in the concentration of free HCl in the unstimulated stomach. The insulin test (9, 10), in which hypoglycemia is induced as a stimulus to vagogenic gastric secretion, has been employed in 21 patients. While the post-vagotomy patterns with this test have generally indicated absence of vagal influence on the stomach, the results in several cases have been equivocal. It is obvious that the intra-gastric reflux of duodenal and jejunal contents through a gastroenterostomy makes the interpretation of the insulin test in Groups III and IV extremely difficult.

### 4. *Roentgenological Changes*

Vagotomy has produced some degree of motor paralysis of the stomach with resulting atony and dilatation and prolonged retention of gastric contents by x-ray in 67% of all the patients studied. Thus, of the 36 patients who had careful pre- and post-operative x-ray studies of gastric function, 24 showed this post-vagotomy gastroplegia, the incidence being lower in the resected group than in the other groups.

It must be pointed out that this figure for gastric paralysis represents x-ray findings and is considerably less than the true incidence of post-vagotomy gastroplegia. The gastric decompression used in the early post-operative period probably contributed to the apparently normal gastric motility seen roentgenologically in 12 patients.

The gastric dilatation and atony characteristically appeared immediately after operation, and, in 10 cases, was first manifested clinically even before the routine post-operative x-rays could be obtained. The usual signs were epigastric discomfort with a palpably enlarged stomach or profuse vomiting of old and foul gastric contents, despite the 48 to 72 hours of continuous suction used routinely in these cases. Typically, this severe loss of function produced by vagotomy, parallels the diminution of secretory activity. In all the satisfactory cases there has been a gradual return of motility and gastric emptying after periods of 6 to 12 months; at present, 6 patients whose stomachs were previously paralyzed have regained complete 5 hour emptying. It is difficult to explain why 3 patients whose post-operative insulin tests showed complete absence of vagal secretory function did not suffer any motor loss whatever.

Of the unsatisfactory cases, 5 have shown persistent gastric retention and dilatation, and 3 of these at reoperation were found to have widely patulous pyloric rings, with only the gastric atony and paralysis to account for their profound gastric retention. The presence of a gastroenterostomy or pyloroplasty seemed to have no effect on the immediate post-vagotomy development of intra-gastric stasis, gastric dilatation and retention, 9 out of 10 cases in this group suffering this alteration of function in the early post-operative period. For the first month the new stoma may not even be seen fluoroscopically to function at all. Once the stomach begins to regain its tone, however, such an artificial opening does prove helpful in hastening the return of motility. The apparent reasons for this in gastroenterostomy are (1) the presence of two exits for food instead of one and (2) the fact that the gastroenterostomy not only has the mechanical advantage of gravity, but may also transmit materials propelled by the weak and incomplete contractions seen in the upper half of the palsied stomach, which would be ineffective in emptying food through the pylorus.

### 5. Failures

The 7 cases with unsatisfactory results following vagotomy are summarized diagrammatically in Table 5. In an evaluation of vagotomy, the two patients whose operations were complicated by splanch-

TABLE 5  
*Unsatisfactory Cases*  
(Failures)

CASE NO.	INDICATION	PROCEDURE	RESULT	OUTCOME
1. J.R.	Perforated gastric	Vagotomy and right splanchnicotomy	Severe retention and recurrent ulcer pain in 10 mo.	Satisfactory after resection
2. J.B.	Pain	Vagotomy and right splanchnicotomy	Recurrent ulcer with pain in 11 mo.	Satisfactory after resection
3. W.A.	Perforated gastric	Vagotomy and repair perforated Cushing ulcer	Died p.o. 14 of meningitis and dural sinus thrombosis	
4. E.R.	Pain	Vagotomy, transthoracic	Severe retention, recurrent ulcer with pain in 5 mo.	Satisfactory after resection
5. J.H.	Pain, gastric	Vagotomy, abdominal, biopsy gastric ulcer	Severe retention, dilatation of stomach in 6 mo.	Satisfactory after gastro-enterostomy
6. L.O.	Perforation	Vagotomy, transthoracic	Fullness, vomiting, gastric atony	Unsatisfactory
7. H.R.	Pain	Vagotomy, transthoracic	Fullness, vomiting, weight loss, gastric atony	Unsatisfactory

nicotomy and the child with Cushing ulcer who died as a result of his brain operation must be excluded. This leaves 4 patients with unsatisfactory results. Two of these have undergone subsequent operations, one for recurrent ulcer, the other for gastroplegia of vagotomy. The other two are suffering with symptoms of this gastric atony and may also need another operation.

## COMMENT

In this series 8 duodenal ulcers have been treated by transthoracic vagotomy performed 10 to 14 days after closure of perforations; 7 of these patients are now symptomless and the 8th is suffering a degree of gastric retention and atony which may need gastroenterostomy. While patients with perforations can expect continued ulcer difficulty which will necessitate subsequent surgery in over one-half the cases, the indication for this seemingly prophylactic procedure may be questioned. The answer to this, of course, may not be known for many years. It is fair to say that this group has done exceedingly well so far. The transthoracic operation has the advantage over other procedures of avoiding the contaminated or potentially contaminated field through which the perforation has been closed. The operation is performed during the same hospital stay and at a time after perforation when the patient is in good condition. The perforation repair facilitates inspection of the lesion and performance of a gastroenterostomy when one is indicated, thus dispelling the usual objection that transthoracic vagotomy does not allow investigation of the lesion being treated. Another advantage of such a scheme is the fact that a complete vagotomy is more readily and certainly accomplished when the esophagus is approached from above rather than below the diaphragm.

It is difficult to explain the apparent disparities between gastric secretory and motor loss in patients following vagotomy. These two functions, in so far as they represent the vagal or cephalic phases of gastric digestion, are characteristically diminished to comparable degrees after operation, but the relationship between these two factors is by no means constant. Three patients who had complete loss of vagal secretory response, as measured by the insulin test, showed no gastric motor abnormality by x-ray. The one patient with recurrence of his ulcer following complete vagotomy continued to show marked symptomatic gastric retention and atony until the time of resection, although a second insulin test performed just before resection gave an equivocal secretory response, suggesting partial reactivation of vagal secretory function. More confusing is one patient with a dramatic result, now followed over 12 months, who shows no impairment of vagal secretion or motility on repeated examination.

Such bizarre results are not common, but they do occur and are

noted or reported by Dragstedt (4, 5, 6), Grimson (7, 8, 14), Moore (11) and others. Undoubtedly more than one factor is responsible for such variations on the general rule. Anatomically incomplete vagus section, the inconsistency of the insulin test and the fact that section of all vagal fibres is not always necessary to effect an apparent cure (14), these three factors play a role in confusing the picture.

Vagotomy performed without any other procedure has produced unsatisfactory results in 4 of the 15 cases. This is a high incidence of failure, 27% within the first year, for any operative procedure for ul-

TABLE 6  
*Overall Results in 41 Patients Followed*

GROUP	NO. OF PATIENTS	SATISFACTORY	UNSATISFACTORY
I (With Splanchnicotomy)	3	1 (33%)	2 (67%)
II (Vagotomy Alone)	16	11 (69%)	5 (31%)
III (With Stoma)	10	10 (100%)	0
IV (With Resection)	12	12 (100%)	0
Total .....	41	34 (83%)	7 (17%)

cer. Of these 4 patients, 2 have since been relieved by subsequent operations on the stomach. The 2 remaining patients should be susceptible to similar relief.

While the role of vagotomy in ulcer therapy is by no means clearly established, we believe that there is sufficient evidence in its favor to justify further clinical trial in selected cases. Occasional failure of vagotomy to heal an ulcer apparently does occur, leaving the patient a candidate for gastric resection. Oftener the gastric motor effect of vagotomy will necessitate a future plastic operation on the stomach, the ulcer having healed satisfactorily. In fairness to the procedure, it must be emphasized that vagotomy does not contraindicate or add

difficulty to further surgical procedures on the stomach. This is a virtue which other operations for ulcer cannot claim.

### SUMMARY

1. Vagotomy, either alone or in conjunction with another operative procedure, has been performed on 43 patients with peptic ulcer. One post-operative death occurred, but this was not related to vagotomy.

2. The clinical results in the 41 patients followed 2 to 24 months have been satisfactory in 34 cases or 83%, and unsatisfactory in 7 cases or 17%. Excluding the one patient who died with complications of craniotomy and 3 patients in whom vagotomy was compli-

TABLE 7  
*Corrected Results in 37 Patients*  
(Omitting 1 pt. with Cushing ulcer and 3 pts. with Splanchnicotomy)

GROUP	NO. OF PATIENTS	SATISFACTORY	UNSATISFACTORY
II (Vagotomy alone)	15	11 (73%)	4 (27%)
III (With Stoma)	10	10 (100%)	0
IV (With Resection)	12	12 (100%)	0
Total .. . . . . .	37	33 (89%)	4 (11%)

cated by simultaneous division of one splanchnic nerve, the corrected results are: 37 patients followed with no mortality; satisfactory results in 33 cases, or 89%, unsatisfactory in 4 cases, or 11%. The 2 patients lost to the follow-up were doing well at the time of discharge.

3. In the satisfactory group, the disappearance of ulcer pain has been most striking and has overshadowed other troublesome, though transient, effects of vagotomy. These in order of frequency, have been: (1) an increase in the number of daily stools with occasional diarrhea; (2) post-prandial fullness and (3) occasional post-operative vomiting, the latter two effects being related to the motor paralysis of the stomach which followed vagotomy in 24 patients or 67% of those studied.

4. In the unsatisfactory group, 2 patients have come to reoperation, one for recurrence of ulceration and one for intractable gastric retention and dilatation due to vagotomy motor paralysis. There are 2 patients in this group who have continued to show uncontrollable signs of severe gastroplegia and who may need gastroenterostomy in the future.

5. All the failures were in patients who had vagotomy without any additional operative procedure, either plastic or ablative, on the stomach itself. The results have been uniformly satisfactory in the 24 patients undergoing some such procedure in addition to vagotomy. Most satisfactory of all have been the 10 patients in Group III who had gastroenterostomy or pyloroplasty along with vagotomy.

#### REFERENCES

1. ALVAREZ, W. C.: Therapeutic Information Please. J. A. M. A. 132: 970. 1946.
2. DRAGSTEDT, L. R., AND OWENS, F. M.: Supra-diaphragmatic Section of the Vagus Nerves in Treatment of Duodenal Ulcer. Proc. Soc. Exper. Biol. & Med. 53: 152, 1943.
3. DRAGSTEDT, L. R., PALMER, W. L., SCHAFER, P. W., AND HODGES, P. C.: Supra-diaphragmatic Section of the Vagus Nerves in the Treatment of Duodenal and Gastric Ulcers, Gastroent. 3: 450, 1944.
4. DRAGSTEDT, L. R.: Vagotomy for Gastroduodenal Ulcer. Ann. Surg. 122: 973, 1945.
5. DRAGSTEDT, L. R., AND SCHAFER, P. W.: Removal of the Vagus Innervation of the Stomach in Gastro-duodenal ulcer. Surg. 17: 742, 1945.
6. DRAGSTEDT, L. R., THORNTON, T. F., AND STORER, E. H.: Supra-diaphragmatic Section of Vague Nerves. J. A. M. A. 130: 764, 1946.
7. GRIMSON, K. S., TAYLOR, H. M., TRENT, J. C., WILSON, D. A., AND HILL, H. C.: Transthoracic Vagotomy. So. Med. J. 99: 460, 1946.
8. GRIMSON, K. S.: Transthoracic Vagotomy. J. A. M. A. 134: 925, 1947.
9. HOLLANDER, F.: The Insulin Test for the Presence of Intact Nerve Fibers after Vagal Operations. Gastroent. 7: 607, 1946.
10. JEMERIN, E. E., HOLLANDER, F., AND WEINSTEIN, V. A.: A Comparison of Insulin and Food as Stimulus for Differentiation of Vagal and Non-vagal Gastric Pouches. Gastroent. 1: 500, 1943.
11. MOORE, F. D., CHAPMAN, N. P., SCULZ, M. D., AND JONES, C. M.: Trans-diaphragmatic Resection of the Vagus Nerves for Peptic Ulcer. N. Eng. J. Med., 234: 241, 1946.
12. PEREIRA, A. DE SOUSA: Blocking of the Splanchnic Nerves and the First Lumbar Sympathetic Ganglion. Arch. Surg. 53: 32, 1946.



13. PAULSON, MOSES, AND GLADSDEN, EUGENE: Symposium on Vagotomy: *Insulin Test and Medical Aspects (In Press)*.
14. RUFFIN, J. M., GRIMSON, K. S., AND SMITH, R. C.: The Effect of Trans-thoracic Vagotomy upon the Clinical Course of Patients with Peptic Ulcer. *Gastroent.* 1: 599, 1946.
15. WEINSTEIN, V. A., ET AL.: Vagotomy in the Therapy of Peptic Ulcer., *Surg., Gynec. and Obst.* 79: 297, 1944.

# MEDICAL ASPECTS OF VAGOTOMY FOR PEPTIC ULCER<sup>1</sup>

## INCLUDING OBSERVATIONS ON THE CLINICAL VALUE OF THE INSULIN TEST AND ON POST-OPERATIVE CRITERIA FOR THE COMPLETENESS OF BILATERAL GASTRIC VAGUS SECTION

MOSES PAULSON<sup>2</sup> AND EUGENE S. GLADSDEN<sup>3</sup>

*From the Department of Medicine (Gastro-Intestinal-Nutrition Division and Laboratories) of the Johns Hopkins University and Hospital, Baltimore, Md.*

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<sup>2</sup> Assistant Professor of Medicine, Physician and Consultant in Gastro-Enterology, Johns Hopkins University and Hospital and its Diagnostic Clinic, respectively.

<sup>3</sup> Mead Johnson Fellow in Medicine.

- VI. Problems associated with Vagotomy
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## INTRODUCTION

In an attempt to modify the acid factor especially in the empty stomach, this being regarded as an important consideration in the etiology of many peptic ulcers, vagotomy was re-introduced and made technically practical by Dragstedt and Owens (2).

Enthusiasm for the procedure in some quarters; the wide interest in medical circles; the natural concern of patients with this disease as to what the new operation has to offer, this being stimulated by descriptions and reports in the lay press; and the possibility that it may materially alter our approach to the problem of management—all of these make it highly advisable not only to evaluate our early experiences with vagotomy in peptic ulcer, but to continue to do so over a period of years, since its final place may not be determinable until then.

## RATIONALE

### *Complete severance of gastric vagal branches for relief of peptic ulcer*

The acid in the gastric secretion is regarded by many as the proximal cause of a large number of peptic ulcers (3,4). As classified by Ivy (5) there are three phases of gastric secretion: gastric, intestinal and cephalic. The first two phases are stimulated and at the same time buffered and diluted by food. The stimulation of the cephalic or psychic phase is independent of food in the stomach and intestines. It is stimulated by the emotions (6) and other sensory phenomena as olfactory, gustatory and ocular impulses which are transmitted to the stomach from the medullary center by way of the vagus nerve, as originally pointed out by Pavlov (7). Under these circumstances the acid may be unbuffered and undiluted, this occurring particularly during the nocturnal hours. Hence, the corrosive action upon the gastric and duodenal mucosa is greater. Therefore, it was hypothe-

sized that complete bilateral section of vagal branches supplying the stomach should abolish this type of secretion hence facilitating ulcer healing and preventing recurrences. For greater detail one is referred to the papers of Dragstedt and his numerous co-workers (2, 4, 8, 9, 10, 11). An important by-product of such a procedure is profound alteration in gastric tone and motility—gastric dilatation and marked diminution of peristalsis or peristaltic inertia.

#### MATERIAL STUDIED

The cases have been divided into four groups:

- I. Vagotomy alone or following previous (10 to 14 days) suturing of a perforated peptic ulcer: total of 14 cases
  - A. Supradiaphragmatic vagotomy: 11 cases
  - B. Infradiaphragmatic vagotomy: 3 cases
- II. Vagotomy, infradiaphragmatically, with stoma: total of 10 cases
  - A. Gastroenterostomy: 8 cases
  - B. Pyloroplasty: 2 cases
- III. Vagotomy, infradiaphragmatically, with subtotal gastrectomy: 15 cases
- IV. Vagotomy with splanchnicectomy

Our data will be somewhat at variance with our surgical colleagues at the Johns Hopkins Hospital because we are eliminating the fourth group—vagotomy with splanchnicectomy. (See Johns and Grose (12)). Theoretically, these systems—sympathetic and parasympathetic—are, in a general way, considered as antagonistic and under normal circumstances are in a state of balance (13), thus tending to further complicate evaluation of vagotomy. Therefore, only the first three groups will be considered.

Group I. *Vagotomy alone or following previous (10 to 14 days) suturing of perforated ulcer (14 cases)*: Six of these patients came to the hospital for treatment of acute perforation of peptic ulcer. These were sutured immediately and after ten days to two weeks a supradiaphragmatic bilateral vagotomy was done. The purpose of the latter operation was to protect against recurring symptoms with or without ulcerations since 50% to 82.5% of this group from three to five years following simple suturing present recurrences and other difficulties (14, 15, 16, 17). Of the other eight cases, three were done infra-

diaphragmatically and five supradiaphragmatically. These eight, (without previous perforation), were done because of persistent complaints over long but varying periods in which medical management was said not to be helpful and which interfered with routine daily activities. The past history was characterized by pain of varying degrees, heart-burn and gnawing, and many had previous episodes of gross hemorrhage. The duration of symptoms was from three months to ten years. Four had ulcer craters, and four manifested deformed caps and gave definite histories of previous attacks of melena.

Group II. *Vagotomy with Stoma (10 cases)*: All cases in this group had infradiaphragmatic vagotomy, two with pyloroplasty and eight with gastroenterostomy. Symptoms varied from one to thirty-seven years, and again pain was the outstanding feature in all but one who had nausea and emesis without retention, but with x-ray evidence of duodenal ulceration. Only three of this group had definite obstruction prior to operation. Here, too, vagotomy was done for relief of the ulcer and the gastroenterostomy or pyloroplasty to prevent the frequent sequelae of the operation: dilatation and retention, which is reported by Grimson and his co-workers (18), and Dragstedt and Schafer (10), and Winkelstein (19) as being of sufficient import in about 20% of cases following simple vagotomy to require additional surgery for its relief.

Group III. *Vagotomy with sub-total gastric resection (15 cases)*: Indications usually were either recurrent hemorrhage or gastric ulcer. Obstruction was encountered in two and pain was pronounced in four prior to operation. Symptoms varied from one to twenty-three years.

## RESULTS

I. *Symptoms: Pain*: Immediately following operation, the above-mentioned symptoms no longer manifested themselves in thirty-six of the thirty-nine patients observed over varying periods up to a year which represents this period of study. The most striking subjective change was complete cessation of pain. In three of the fourteen cases in Group I, distress recurred within a few months after operation; in one it was associated with marked retention and relieved by gastroenterostomy; in another, evidence of ulceration as well as distressing fullness persisted after four months and was satisfactorily treated by

subtotal gastric resection; and in the third case, no further studies were permitted by the patient to account for the return of pain, nausea, emesis, loss of weight and tarry stools.

*Fullness:* All of the cases in Group I (vagotomy without pyloroplasty, gastroenterostomy or sub-total gastrectomy) complained of varying degrees of fullness. In only two instances, thought to be due to gastric paralysis following operation, was further surgical intervention necessary. However, in the others, as time went on, this symptom became much less pronounced.

*Nausea and emesis:* This occurred only on rare occasions for short periods after hospital discharge. Often it was regurgitation rather than emesis. This was ascribed to the gastric dilatation, atony and peristaltic inertia.<sup>4</sup> These complaints eventually disappeared.

*Appetite:* All experienced hunger sensations. This resulted in a remarkable gain in weight in most instances.

*Defecation:* A change in bowel habit was noted. Previous to operation, all were constipated. Following operation, nearly all had one to three well formed movements daily. In a few, shortly after operation, there was diarrhoea for brief periods which was readily controlled by simple measures, or was relieved spontaneously. After a few months, this was not present in any case.

*Ability to work:* All patients (except the three cases presenting post-operative problems in Group I referred to above) spoke of increasing vigor and their ability to do a full day's work.

*Chest pains at operative site:* In those in whom transthoracic vagotomies were done, there was pain at the site of the chest incision which usually subsided in a few weeks.

II. *Signs: X-rays:* A characteristic phenomenon of bilateral vagotomy is gastric dilatation, atony and peristaltic diminution or inertia. All of these factors are not always present and they are not always interrelated. Sometimes there is dilatation and the motility is rela-

<sup>4</sup> Control of this problem was facilitated by the use of Urethane of B methylcholine chloride (Urecholine-Merck). A 5 mgm. tablet is given three times daily, one hour before eating. This has been administered indefinitely without untoward reactions. Apparently this cholinergic agent stimulates vagal nerve endings and results in better gastric emptying. We are indebted to Dr. Augustus Gibson of Merck & Company, Inc., Rahway, N. J., for his cooperation.

tively satisfactory. The reverse situation is also seen. A few almost immediately following vagotomy manifest normal tone and motor function. In many, the stomach tends to assume normal tonicity and activity by the end of the first post-operative year confirming the observations of Moore, Chapman, Schulz and Jones (20). Details are also enumerated by Johns and Grose (12).

*Gastroscopic appearances:* In those without gastroenterostomy and sub-total resection, there is virtually no change in the gastric mucosal pattern. However, the stomach is less active than that usually encountered. The antrum appears relatively patulous and there is lessened peristaltic activity; this might be expected in view of interference with the vagal innervation of the stomach. The unusual finding is the tendency of the pyloric sphincter to remain more or less open except when a peristaltic wave rushes over it momentarily following which it reverts to relative patulousness. This is not unlike that seen in an occasional case without vagotomy but which has been saturated with an anti-spasmodic. However, these findings are contrary to what is noted—gastroscopically—in the untreated and non-vagotomized stomach where the antrum is of better tone and the sphincter seems closed for the most part, visualization of the latter being difficult and momentary. To what extent the resumption of normal tone of the antrum and pyloric sphincter is to be seen gastroscopically in those with vagotomy at a later date, remains to be determined. Incidentally, these gastroscopic findings following vagotomy are in line with those which surgeons encountered during re-operation following previous vagotomy. In vagotomy with gastroenterostomy, some negligible mucosal changes may be seen. But here, too, the antrum and the pyloric sphincter are as above described. In sub-total gastrectomy, the oedema, thickening and nodular-like hypertrophy, with or without erythema and friability, as noted in similar instances without vagotomy (21) are also seen (see Figs. 1 and 2). As far as we know these findings have not been recorded elsewhere; this problem will be discussed further in a subsequent communication.

III. *Complications:* To date—within one year following operation—only three presented problems. These were among fourteen in Group I already referred to.

IV. *Disturbance of other organs:* An attempt was made to study the



FIG. 1 Nine months after vagotomy and subtotal gastric resection: nodularity, hypertrophy, friability and erosion of residual stomach as well as thickening with irregularity of contiguous jejunal folds. (Case Y).



FIG. 2 Three months after vagotomy and subtotal gastric resection. oedema, erythema, ready friability and bleeding of the residual stomach with a thinning out of contiguous jejunal folds (Case X).



TABLE 1

*Pancreatic Ferments in the Serum Before and After Vagotomy*

CASE	OPERATION	AMYLASE*			LIPASE†		
		Before operation	After operation		Before operation	After operation	
		mgm %	mgm %	Days	cc	cc	Days
1 I S	Subdiaphragmatic vagotomy with gastroenterostomy	—	—	—	0	0.3	17
2 L V	Suture of perforation followed by trans thoracic vagotomy	342	366	11	—	—	—
3 W M R	Subdiaphragmatic vagotomy with sub total resection	198	445	9	—	—	—
4 W L	Subdiaphragmatic vagotomy with sub total resection	202	132	9	0	0.4	13
5 J D	Suture of perforation followed by trans thoracic vagotomy	194	162	9	0.4	0	26
6 H R	Subdiaphragmatic vagotomy	122	302	10	—	—	—
7 P T	Suture of perforation followed by trans thoracic vagotomy	230	132	7	0	0.1	7
8 R W	Suture of perforation followed by trans thoracic vagotomy	248	127	—	—	—	—
9 W C	Subdiaphragmatic vagotomy with gastroenterostomy	254	232	—	—	—	—
10 F M	Subdiaphragmatic vagotomy	—	—	—	0.7	0	—

\* Myers, V C, Freed, A H, Rosinski, E A. J Biol Chem, 154.: 39, 1944. Normal values—90-280 mgm % reducing substance

† Todd, J C, and Sanford, A H. Clinical Diagnosis by Laboratory Methods. Philadelphia and London, W B Saunders Co. Ed X, 1945, p. 435. Normal values—0-1.2 cc N/20 NaOH

influence of vagotomy on pancreatic ferments (see Table 1). In two cases (3 and 6) elevations in serum amylase occurred subsequent to this operation, and in one case (case 2) this enzyme was elevated both before and after vagotomy. In five, this determination was within normal limits before and after operation. Serum lipase was normal in all (five cases) before and after operation. No conclusions could be reached here.

The change in bowel habit which was thought to have borne some relationship to alteration in pancreatic function by virtue of vagotomy, was indistinguishable in those with normal from those with abnormal serum amylase values. Changes in organs other than those already mentioned have not been noted or recorded to date.

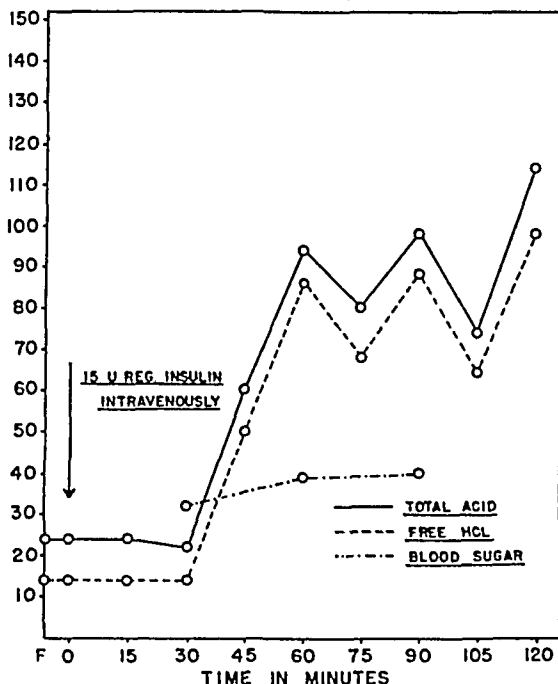
*V. Insulin test:* This test was suggested by the work of Raholm (22), Ihre (23) and Babkin (24). Its purpose is to determine the completeness of severance of vagal fibres supplying the stomach. A critical analysis of it appears under "Comment" elsewhere.

It is based on the generally accepted physiological fact that hypoglycemia induced by insulin administration stimulates the vagal center in the medulla, and in turn results in stimulating both the motor (26) and secretory (22, 27) mechanisms of the stomach by way of the vagi. This phenomenon is noted in the human stomach with intact vagi (see Graph I). The resulting stimulation of gastric secretion can be abolished upon exhibition of atropine (30) or after glucose administration or epinephrine (25). The ability to detect the presence of even a few uncut vagal fibres innervating the stomach in gastric pouches of dogs by this method was shown by Jemerin, Hollander, and Weinstein (28). Based on these data, Weinstein, Colp, Hollander and Jemerin (29), and Hollander (25) devised a clinical test to determine whether any uncut branches supplying the stomach remained after bilateral vagotomy.

In order to obtain a gastric secretory response to insulin-induced hypoglycemia transmitted by the vagus nerves, the blood sugar level must be reduced to 50 mg per 100 cc. or less. After simple vagotomy operations this is usually accomplished by 15 units of regular (unmodified) insulin given intravenously, and where vagotomy is accompanied by a gastroenterostomy or subtotal gastrectomy, this is increased to 20 units in the hope of obtaining a greater outpouring of acid to off-set neutralization by regurgitated intestinal contents in both gastroenterostomy and subtotal gastrectomy and to get the maximum response from a re-

duced acid secretory membrane after the latter operation. After an over night fast, a Levine tube is introduced into the stomach and all of the gastric contents aspirated, measured and titrated for free and total acidity. Fifteen (or twenty) units of regular (unmodified) insulin is given intravenously and the time recorded. At fifteen minute intervals for two hours the stomach contents are completely emptied and the free and total acidity of each specimen are determined. Blood

H. R. M W 46 407633 INSULIN TEST  
PRE-OPERATIVE VAGOTOMY



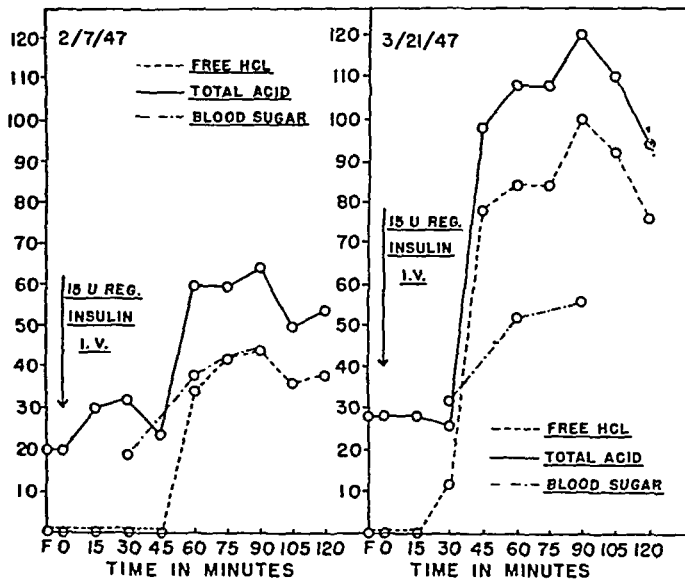
GRAPH I

samples are taken prior to and at one-half hour intervals for one and a half hours after insulin administration.

The response is regarded as positive if there is an increase in gastric acidity after insulin administration (Graph II). This is thought to indicate that vagal innervation has been incompletely interrupted. A negative response (Graph III) is one in which there is no appreciable change in gastric acidity following injection of insulin. This is generally interpreted as evidencing complete interruption of vagal innervation to the stomach.

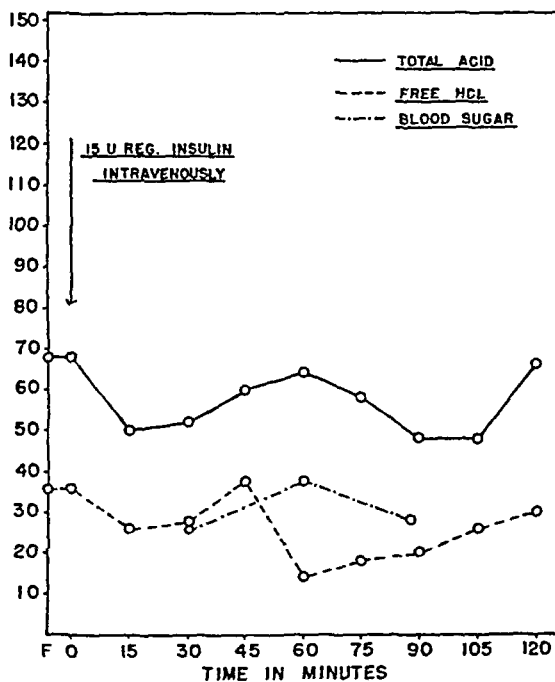
Gastric retention and stasis of long duration is common after vagotomy. This

R.M. M C 38 225705 INSULIN TEST  
POST-OPERATIVE TRANS-THORACIC VAGOTOMY



GRAPH II

H. R. M W 46 407633 INSULIN TEST  
POST-OPERATIVE VAGOTOMY



makes it difficult to secure uncontaminated gastric juice. We have solved this problem by giving only clear fluids for twenty-four hours prior to the test and nothing by mouth after six p.m. of the preceding night. Drugs influencing secretion or motility are also interdicted for a day prior to the test. Smoking is forbidden.

Eleven of fourteen cases with simple vagotomies (Group I) had insulin tests performed post-operatively. Three gave positive tests; the clinical response was satisfactory in two and unsatisfactory in one where a subtotal gastrectomy had to be performed later because of recurrence of ulceration. Five responded with negative tests; the clinical results in all were considered generally satisfactory. In two, the test was equivocal but with clinically satisfactory results in both to date. In one the test was unsatisfactory as was the clinical response.

In group II (bilateral vagotomy with pyloroplasty or gastroenterostomy) seven post-operative insulin tests were done; two were positive and five were negative. The clinical response was satisfactory in all.

In group III (bilateral vagotomy with subtotal gastrectomy), all were symptomless even though one of the three post-operative insulin tests was positive.

VI. *Dietotherapy*: Only where subtotal gastrectomy is performed in addition to vagotomy, are patients instructed to partake of six bland feedings daily because of the smallness of the residual gastric pouch. In the other groups there are no dietary restrictions.

VII. *Pharmacotherapy*: None was given in any instance save in an occasional bout of diarrhoea when an antispasmodic and sedative were administered temporarily.

#### COMMENT

From a medical standpoint, what has been accomplished in a period of observation of a year? Final answer cannot be had because many sequelae and complications following gastric surgery appear after many years. Therefore, adequate evaluation is impossible for at least 5 to 10 years. However, it is significant that some of the advantages and disadvantages following vagotomy recorded here have appeared in about the same relationship in the experiences of Grimson

and his co-workers (18) after one and a half years, Moore, Chapman, Shulz and Jones (20, 31) up to two years, and Smith, Ruffin and Baylin (32) up to twenty-seven months.

It is to be noted that our interpretations may be at variance with our surgical colleagues (12) with whom we have studied and observed the same material. This arises from differences in approach and viewpoint.

It is quite clear to us that the most striking relief afforded by this operation is freedom from pain, heartburn, gnawing and a vague sense of nausea. Fullness is present in most instances but tends to disappear and is rarely troublesome. Patients with gastroenterostomy or pyloroplasty have fullness at the outset but to a lesser extent. These patients eat everything, three time daily, and are without psycho-, dieto-, or pharmacotherapies. Those with sub-total gastrectomy are directed to eat smaller portions of a smooth dietary about six times daily at the outset. This is necessary to avoid fullness which is greater here than in gastroenterostomy due to diminished gastric capacity. Subsequently, patients reduce feedings voluntarily to about three to five times daily.

The relative bowel frequency among all groups—one to three daily defecations in lieu of constipation—usually requires no treatment, is welcomed by the patient, and for the present remains unexplained.

In some instances severe emotional disturbances which prior to operation were usually followed by distress are experienced now without discomfort. Patients seem enthusiastic about the procedure.

#### *Factors Indicating Complete Bilateral Section of Gastric Vagal Branches*

To date, the *sine qua non* for success to follow the operation of bilateral vagotomy, is thought to be complete severance of vagal fibres supplying the stomach. There is a striking difference of opinion as to the distribution of the gastric branches of the vagus nerve. Bradley, Small, Wilson and Walters (33) indicate a fairly constant pattern of arborization of the vagi over the lower oesophagus in 90% of cases and therefore believe the infradiaphragmatic approach is adequate. At the same time Miller and Davis (34) demonstrate numerous branches and a variety of patterns and hence advocate a supra-diaphrag-

matic section. Rienhoff (35) has pointed out that in his denervation of the lungs for asthma, fibres of the vagus nerve enter the sympathetic chain in the cervical region and are carried along in this structure to the celiac plexus from which they are distributed to the stomach as parasympathetic fibres. In addition, Osler Abbott (36) comments on the frequency with which the right vagus nerve breaks up into multiple filaments at a high level, some of which reach the stomach after a considerable course in the muscularis of the oesophagus. Thus, complete severance of vagal branches involving the stomach becomes a problem. At present, the clinician can only employ the following to determine complete section: 1) symptoms; 2) the insulin test; 3) the degree of gastric acidity and the volume of nocturnal secretion; and 4) gastric tone and motility.

1. *Symptoms*: In this entire group of cases symptoms are an unreliable guide to determine completeness of vagotomy. If the insulin test is an index of complete severance, then our results would indicate that certainly within the first year, there is no difference in the relief of symptoms between those who would appear to have complete vagal section as contrasted with those with incompleteness. In all but three instances were they relieved immediately after their operation of all distressing manifestations regardless of their responses to the test.

2. *Volume of gastric secretion*: After vagotomy there is a definite decrease (50% or more) in volume of nocturnal secretion as compared with pre-operative levels (4). In general there is a reduction of free hydrochloric acid, but this is not as uniform as the volumetric change. However, as time goes on, apparently the volume tends to resume its usual pre-operative level (20). Thus, diminished gastric secretory volume in itself can not be used as a gauge indicating complete and permanent severance of gastric vagal fibres.

It remains to be proved that an increase or decrease in nocturnal gastric secretory volume is significantly related to a positive or negative insulin test indicating partial or complete vagal section, respectively. We have evidence in two cases showing that in the face of adequate insulin-induced hypoglycemia, reduction in volume accompanies an equivocal as well as a positive test. On the other hand, a repetitiously negative insulin test may be present even though there is a gradually increasing secretory volume. Apparently gastric autonomy accounts

for this (20). Thus, again, it would appear that the decrease or increase in volume of gastric secretion per se is *no* index of the completeness of vagal section.

3. *Alterations in gastric tone and motility*: Section of the vagus nerves—complete or incomplete as judged by the insulin test—will in the majority result in profound changes in gastric tone and motility which, in large numbers, tend to revert to normality within a year. There are also some cases with apparent complete section and little or no changes in tone and motility. Thus, these changes in themselves cannot be used as a measure of completeness of vagal section.

4. *Palmer acid test*: The introduction of 300 cc. of  $\frac{1}{2}\%$  hydrochloric acid into the stomach of patients with peptic ulcer causes pain (positive response). The results obtained in peptic ulcer patients following vagotomy are contradictory. For instance, Smith, Ruffin and Baylin (32) maintain that the test is negative (no pain after the introduction of acid into the stomach) following complete bilateral vagotomy; Dragstedt (37) says patients have pain following the introduction of acid after vagotomy and uses these data to indicate that the operation does not produce anaesthesia by cutting the vagus nerves. In consequence, this cannot be used to establish complete vagal section at the present time.

5. *Insulin test*: The rationale and criteria of positive and negative insulin tests as well as their meanings have already been pointed out. The insulin test, in our hands, is particularly difficult of interpretation in those either with sub-total gastrectomies or gastroenterostomies. Dilution and neutralization of gastric secretion by small intestinal contents, and, in addition, the paucity of residual secreting gastric tissue after sub-total gastrectomy, despite larger doses of intravenous insulin, account for this. Thus, the insulin test is more satisfactory in stomachs with simple vagotomies.

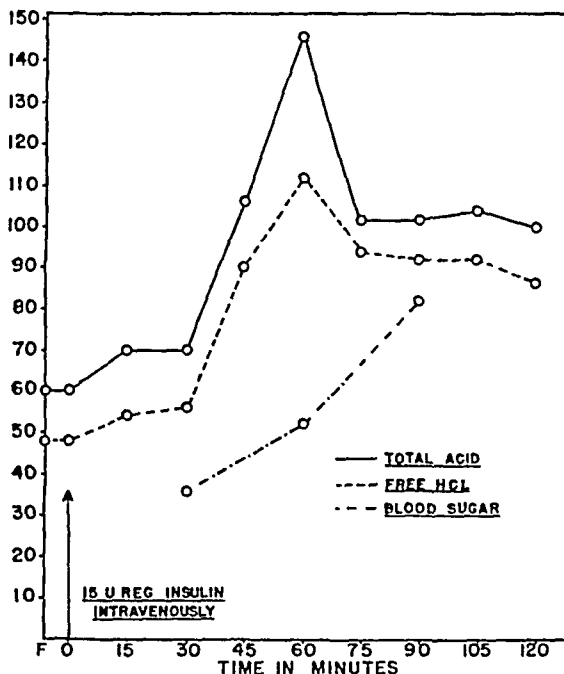
If the insulin test is clinically accurate, is it essential to cut all of the gastric vagal branches when freedom from disturbing symptoms, alterations in gastric tone, motility and secretion occur whether the test is positive, equivocal or negative? If these changes take place without the inviolate occurrence of complete severance as determined by the insulin test, may not the original premise of *total* ablation of all vagal branches to the stomach as a condition inherent to the success



of the operation be in error? Anatomically, there is a question whether all the vagal fibres to the stomach can always be interrupted (33, 34, 35, 36).

On the other hand, may not the interpretations to responses to the insulin test in human beings be questioned at this time? Three circumstances suggest this: a) our experience with a negative test

F. McN. M W 44 407354 INSULIN TEST  
PRE-OPERATIVE VAGOTOMY

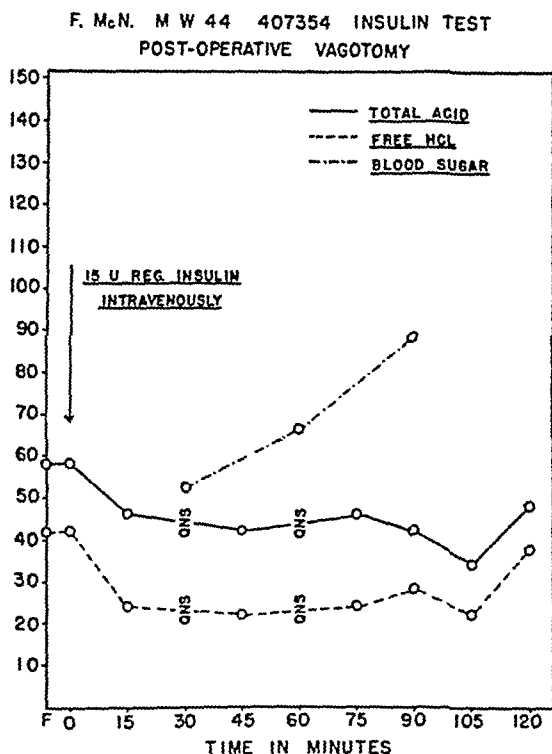


GRAPH IV

in one instance when the surgeon was certain he had not cut all of the fibres since the operation had to be terminated abruptly due to the sudden collapse of the patient (see Graphs IV and V). Criticism may be offered that the insulin induced hypoglycemia in this case measured 52 mgs. of glucose per 100 cc. instead of 50 mgs. the upper limit insuring adequate hypoglycemia. However, another technician could have read this just as easily as 50 mgs%. This patient had complete

symptomatic relief and changes in gastric tone and motility noted in those regarded as having had complete severance of vagal fibres.

b) As indicated above, in the majority there are no determinable subjective or objective differences in our hands within the first year after vagotomy operations, in those with positive, equivocal and negative tests. It remains to be established that a significant number with



GRAPH V

positive insulin tests, (said to indicate incomplete section thereby implying an unsatisfactory operative outcome), eventually fare worse than those with persistently negative tests, assuming, of course, that the tests have been properly performed.

c) While the insulin test is based on sound physiological grounds, and while it may adequately measure complete vagal section in dogs with gastric pouches, these in themselves do not necessarily mean that

complete section of the vagi can be ascertained just as effectively by this method in the more complicated human machanism. As already discussed, it may be that from a practical clinical standpoint such complete vagal severence is unnecessary, and indeed may be impossible in some instances. If so, how is the insulin test to be used in interpretation between total, adequate and inadequate vagal section? This is not to say that the insulin test should not be performed. It should be continued in the hope of eventually evaluating it properly from a clinical standpoint. It should be repeated over varying periods when negative, for if the test should become positive it would probably indicate sufficient regeneration of vagal tissue of possible clinical importance. However, it remains to be proved that a single positive insulin test in itself indicates inadequate and hence unsatisfactory vagal section from a clinical standpoint.

6. *Other criteria in establishing adequate vagal section:* They are the continued absence of pain, gnawing and heart-burn, the absence of objective evidence of gross or occult bleeding, recurring ulceration or fistula formation. The history, x-rays, determinations of gross or occult blood in gastric aspirates and in feces, as well as gastroscopy, are the methods to be employed. Serial insulin tests, particularly in those with stomachs capable of secreting adequate acids, may eventually become informative for reasons given above.

VI. *Problems Associated with Vagotomy:* A. *Pain:* The complete relief of pain afforded by vagotomy is not an unmitigated blessing. The clinician may have to develop other attitudes and new approaches to recognize recurrent ulceration, perforation and obstruction in patients whose pain sense has been obliterated or obtunded following vagal section.

B. *Gastritis—jejunitis:* Comparing our gastroscopic experiences in sub-total gastrectomy without vagotomy, it is now possible to say that bilateral vagotomy does not protect the patient from developing oedema, erosion, hypertrophy and friability in the residual gastric pouch and the attached jejunum. The former group often present themselves with various and sundry complaints and respond readily to simple measures. However, those with vagotomy and sub-total gastrectomy, during their period of observation (first post-operative year), are without complaints and appear to be in no need of any medical

management. Two questions arise. 1) Is this painlessness desirable? 2) Will these changes eventually lead to recurring hemorrhage and ulceration despite vagotomy?

VII. *Indications for vagotomy*: By virtue of our experiences to date, the following is our method of approach with regard to the selection of cases for vagotomy at this time:

1. *Intractable pain*: If intractable pain is to be defined as continuous, unremitting distress uninfluenced by combined psycho, dieto and pharmacotherapies in those with uncomplicated duodenal ulcer (ulcer without evidence of previous perforation, gross hemorrhage or obstruction), then we must report that we have never encountered this phenomenon. Apparent intractability of distress under these circumstances is usually the result of the patient's failure to follow a prescribed routine or the inadequacy of the clinician in management. To relieve this type of patient from his complaints by merely performing vagotomy teaches him nothing. The manner in which his personality reacts to life's situations may sooner or later express itself in other organs and in other forms. Besides, this type of patient is being subjected to a 20% chance of having additional gastric surgery performed in consequence of certain sequelae and complications of vagotomy to which references have been made. Therefore, vagotomy, or any type of surgery, is not indicated—in our humble opinion—in this type of problem.

2. *Complications*: a) *Acute perforation*: The fact that 50% to 75% of cases manifesting acute perforation are said to return with varying types of distress in three to five years following suturing, makes the addition of vagotomy 10 to 14 days following this procedure a welcome addition in the hope of preventing recurrences. It must be remembered that not all of the 50% to 75% of those returning with difficulties present surgical problems. However, at the present time we are inclined to urge supradiaphragmatic vagotomy within two weeks following suturing for acute perforation.

b) *Recurring hemorrhage*: If peptic ulcer can be demonstrated as the cause and it is without obstruction, and operation is decided upon later to prevent subsequent bleeding, then simple vagotomy may be undertaken in preference to the removal of about 75% to 90% of the stomach—a sub-total gastrectomy—an operation which can be undertaken at a later date if necessary.

c) *Obstruction*: Whenever a gastroenterostomy or a sub-total gastrectomy is to be done for obstruction due to cicatricial tissue, then vagotomy may also be performed in the hope of preventing recurring ulceration, particularly in the region of the new stoma.

#### VIII. SUMMARY

I. A total of 39 cases were evaluated from the medical standpoint within a year following attempted complete bilateral section of gastric vagal fibres for relief of peptic ulcer and its complications.

II. The rationale of the procedure was discussed.

III. *The striking results were the relief from pain, heart-burn, gnawing and nausea in all but three, two of whom required further surgery.*

IV. X-rays revealed disturbances in tone and motility in the large majority, which tended to resume some semblance of normality within a year.

V. Gastrosopic findings are recorded.

VI. The insulin test is critically analyzed.

VII. The criteria for establishing adequate vagal section post-operatively is presented since complete bilateral section of vagal gastric branches may not always be obtainable and such may not always be necessary.

VIII. The obliteration or obtunding of pain following this operation is not an unmitigated blessing.

IX. Indications for vagotomy are discussed.

#### REFERENCES

1. BOCKUS, H. LE R.: *Gastro-enterology*, 3 vol. Philadelphia, W. B. Saunders Co., 1943-1946. Vol. 1, p. 499.
2. DRAGSTEDT, L. R., AND OWENS, F. M., JR.: *Supradiaphragmatic Section of Vagus Nerves in Treatment of Duodenal Ulcer*. *Proc. Soc. Exper. Biol. and Med.*, **53**: 152, 1943.
3. EDITORIAL: *The Acid Factor in Peptic Ulcer*. *J. A. M. A.*, **101**: 857-858, 1933.
4. DRAGSTEDT, L. R., PALMER, W. L., SCHAFER, P. W., AND HODGES, P. C.: *Supradiaphragmatic Section of the Vagus Nerves in the Treatment of Duodenal and Gastric Ulcers*. *Gastroenterol.*, **3**: 450-462, 1944.
5. IVY, A. C.: *Contributions to the Physiology of the Stomach*. IX. *The Causes of Gastric Secretion; Their Practical Significance and the Mechanisms Concerned*. *J. A. M. A.*, **85**: 877-880, 1925.

6. WOLF, S., AND WOLFF, H. G.: Human Gastric Function. An Experimental Study of a Man and his Stomach. New York, Oxford University Press, 1943.
7. PAVLOV, I. P.: The Work of the Digestive Glands. London, Charles Griffen, 1910, p. 53.
8. DRAGSTEDT, L. R.: Some Physiologic Principles Involved in the Surgical Treatment of Gastric and Duodenal Ulcer. *Ann. Surg.*, 102: 563-580, 1935.
9. DRAGSTEDT, L. R.: Pathogenesis of Gastroduodenal Ulcer. *Arch. Surg.*, 44: 438-451, 1942.
10. DRAGSTEDT, L. R., AND SCHAFER, P. W.: Removal of the Vagus Innervation of the Stomach in Gastroduodenal Ulcer. *Surgery*, 17: 742-749, 1945.
11. DRAGSTEDT, L. R.: Editorial—Section of the Vagus Nerves to the Stomach in the Treatment of Peptic Ulcer. *Surg., Gynec., and Obst.*, 83: 547, 1946.
12. JOHNS, T. N. P., AND GROSE, W.: Symposium on Vagotomy: II. Early Surgical Results in Forty-three Cases. *Bull. Johns Hopkins Hospital*, 81: 92-106, 1947.
13. KUNTZ, A.: A Textbook of Neuroanatomy. 4th Ed. Lea and Febiger, Philadelphia, 1945. P. 319.
14. WILLIAMS, H., AND WALSH, C. H.: Treatment of Perforated Peptic Ulcer. *Lancet*, 1: 9, 1930.
15. SALLICK, M. A.: Late Results in Acute Perforated Peptic Ulcer Treated by Simple Closure. *Ann. Surg.*, 104: 853-863, 1936.
16. HARRISON, C., AND COOPER, F. W.: Immediate and Late Results of Perforation of Peptic Ulcer. *Ann. Surg.*, 116: 194-199, 1942.
17. See Reference 1. P. 517.
18. GRIMSON, K. S., TAYLOR, H. M., TRENT, J. C., WILSON, O. A., AND HILL, H. C.: The Effect of Transthoracic Vagotomy upon the Functions of the Stomach and upon the Early Clinical Course of Patients with Peptic Ulcer. *South. Med. J.*, 39: 460, 1946.
19. WINKELSTEIN, A.: Discussion on Symposium on Peptic Ulcer with Particular Reference to Vagotomy. *Gastroenterol.*, 7: 616, 1946.
20. MOORE, F. O., CHAPMAN, W. P., SCHULZ, M. O., AND JONES, C. P.: Resection of Vagus Nerves in Peptic Ulcer. *J. A. M. A.*, 133: 741, 1947.
21. PAULSON, M., AND GLADSDEN, E. S.: Data to be Published.
22. RAHOLM, K.: Clinical Investigations into the Effect of Intravenous Injection of Insulin. IX. Gastric Secretion in Normal Individuals. *Acta Med. Scandinav.*, 73: 472, 1930.
23. IHRE, B. J. E.: Human Gastric Secretion. London, Oxford Univ. Press, 1939.
24. BABKIN, B. P.: Secretory Mechanism of the Digestive Glands. New York, Paul Hoeber, Inc., 1944.

25. HOLLANDER, F.: Insulin Test for Intact Nerve Fibers. *Gastroenterol.*, 7: 607, 1946.
26. BULATAO, G., AND CARLSON, A. J.: The Relation of the Blood Sugar to the Gastric Hunger Contractions. *Am. J. Physiol.*, 68: 148, 1924.
27. KALK, H., AND MEYER, P. F.: Blutzuckerspiegel und Magensekretion. *Ztschr. f. klin. Med.*, 120: 692, 1932.
28. JEMERIN, E. E., HOLLANDER, F., AND WEINSTEIN, V. A.: A Comparison of Insulin and Food as Stimuli for Differentiation of Vagal and Non-Vagal Gastric Pouches. *Gastroenterol.*, 1: 500, 1943.
29. WEINSTEIN, V. A., COLP, R., HOLLANDER, F., AND JEMERIN, E. E.: Vagotomy in the Therapy of Peptic Ulcer. *Surg., Gynec. and Obst.*, 79: 297-305, 1944.
30. BABKIN, B. P.: Testing of the Secretory Activity of the Gastric Glands in Man by Means of Histamine and Insulin. *Am. J. Dig. Dis.*, 5: 753, 1939.
31. MOORE, F. D., CHAPMAN, W. P., SCHULZ, M. D., AND JONES, C. P.: Transdiaphragmatic Resection of the Vagus Nerves for Peptic Ulcer. *New England J. Med.*, 234: 241-251, 1946.
32. SMITH, R. C., RUFFIN, J. M., AND BAYLIN, G. J.: The Effect of Transthoracic Vagus Resection upon Patients with Peptic Ulcer. *South. Med. J.*, 40: 1-10, 1947.
33. BRADLEY, W. F., SMALL, J. T., WILSON, S. W., AND WALTERS, W.: Anatomic Considerations of Gastric Neurectomy. *J. A. M. A.*, 133: 459, 1947.
34. MILLER, E. M., and DAVIS, C. B., JR.: An Anatomic Study of the Vagus Nerves. *J. A. M. A.*, 133: 461, 1947.
35. RIENHOFF, W.: Discussion, see Reference 18.
36. ABBOTT, O.: Discussion, see Reference 32.
37. DRAGSTEDT, L. R.: Section of the Vagus Nerves to the Stomach in the Treatment of Peptic Ulcer. *Surgery*, 21: 144, 1947.

## DISCUSSION OF THREE PAPERS OF SYMPOSIUM ON VAGOTOMY FOR PEPTIC ULCER

*Dr. A. M. Harvey:* I would like to ask Dr. Grose if they have used any of the cholinergic drugs such as urocholine for their effect on motility. The vagus is a cholinergic nerve and after it is cut the stomach becomes very susceptible to the stimulating action of these drugs, and they have been found useful in controlling that difficulty which follows vagotomy.

*Dr. William Grose:* Dr. Harvey, we have had experience with the drug (urocholine). The drug has been used only while the patients were still in the hospital. In two such patients gastric motility was perfectly normal during the post-operative stay in the hospital and the patients were doing well on discharge when the drug was discontinued. One patient also showed normal gastric motility and emptying. The patient was on the drug for a very short period of time, developed side effects, and the drug was discontinued. Two days later there was seen huge gastric dilation which has persisted.

*Dr. John Eager Howard:* Are there any further questions?

*Dr. George Finney:* I would like to offer one thing for what it is worth. I don't believe that we can throw all operations on the stomach, plus vagotomy, in together and get any true evaluation of the possibilities of vagotomy or its bad effects. I would like to ask Dr. Grose about those four patients that he said were bad results from the surgical standpoint. I would like to know what other surgery was done. I would also like to know whether the vagotomy was done from above the diaphragm or below, because in my very limited experience I can't help but feel that those are very important factors. I think we ought to group our supra-diaphragmatic cases together, namely those who have no other surgery, because I believe there is much more possibility of getting all the vagal fibers by going through the chest. Then again I think we ought to group our cases done from below the diaphragm without any other procedure except vagotomy in another group and, in a third group, those where other surgery is done, plus vagotomy. Other surgeons have noted that there is a great difference many times in the results, both objective and from the patient's standpoint. I



would also like to mention two cases which were very interesting and see if anyone could perhaps give the explanation of the findings. Both cases had very definite duodenal ulcers which were quite deep. Films were made just before operation, and then were made, one in nine days and the other in ten days post-operative—done by the same X-ray man, and at this time ulcers could not be demonstrated in either of these cases. Now I can't believe that those ulcers healed in that short period after vagotomy, but I wonder what part the lack of spasm plays, and also its part in the relief of pain.

*Dr. William Grose:* Dr. Finney, one of those four cases was a man done through the chest. The operator thought the division of nerve was complete. The insulin test done after operation seemed to show a complete vagotomy. He did well for several months and then began to develop fullness and later began to develop pain which he said was similar to his original ulcer pain. He was re-explored and a definite active duodenal ulcer was found, typical grossly and microscopically. That was one failure. The second was a man who had an operation for exploration for a gastric ulcer which was biopsied and was benign on frozen section. Because he was in very poor shape, only a vagotomy was done. That was below the diaphragm. He went from good to bad, to good to bad, shape over a period of months and finally was reexplored. The ulcer seemed to have healed. He simply had dilation of the stomach, atony so severe that he could not get food through a patulous pylorus. The other two cases are two that had vagotomies and who are suffering from fullness and repeated vomiting.

As to the disappearance of duodenal ulcer very shortly after vagotomy, I can only say that it is so very difficult to get barium to fill the duodenum of these people after vagotomy it seems to me hard to evaluate the x-ray evidence of healing of an ulcer. At least, the stomach itself doesn't push barium through very satisfactorily and I suppose it would be hard to manipulate it so as to fill well the duodenum at the site of the ulcer.

*(Adjournment)*

# THE EXPERIMENTAL INVESTIGATION OF PEPTIC ULCER\*

EDWARD M. HANRAHAN

*From the Department of Surgery of the Johns Hopkins University and Hospital and from the Surgical Hunterian Laboratory*

Much of our knowledge of the pathogenesis and pathologic anatomy of peptic ulcer is based on the observations of Cruveilhier (1), Rokitsky (2) and Cohnheim (3). In fact little can be added to Welch's (4) account of the subject in 1885. With more accurate medical and surgical diagnosis we have perhaps a clearer conception of its clinical incidence and psychosomatic basis. It occurs almost exclusively in man, and in about 95% of cases it occurs in a limited portion of the stomach and duodenum, that is, along the lesser curvature more often posterior than anterior, in the antrum and in the duodenum rarely below the ampulla. It is far more common in the white race and in men more than women. Its period of onset is the period of increasing ambition and responsibilities. Once an individual has shown himself vulnerable to peptic ulcer, clinical experience suggests that it may be a lifetime affliction with periods of exacerbation and remission; that the tendency to heal is opposed by some derangement, the complete nature of which we are ignorant.

Most experimental ulcers are thought to be the result of the action of gastric juice on injured mucosa. Ivy (5) points out, as did MacCallum (6), that even these two factors, injury and digestion, if unaccompanied by continuance of the underlying cause are probably insufficient to prevent healing. Berg (7) recently expressed the opinion that penetrating lesions may not be preceded by a mucosal defect but are caused by a single factor which simultaneously affects all the layers of the gastric walls.

One example of chronic ulcer produced by surgical alteration of gastric function is the jejunal ulcer which sometimes occurs after gastro-jejunostomy in man and in animals. Following the demonstration by

\* Although the paper was not given as part of the Symposium on Vagotomy For Peptic Ulcer, it is included here because of its pertinency to the subject at hand. The original Max Brödel drawings are herein published for the first time.  
—Editor.

Exalto (8) that such jejunal ulcers may be regularly produced by performing gastrojejunostomy with pyloric exclusion and draining duodenal contents into the proximal loop of the cecum, Mann (9, 10, 12) and his associates developed and modified the procedure and made exhaustive studies of this type of ulcer. Their method, which Morton (13, 15) called surgical duodenal drainage, has refocused attention on the part played by normal intact pyloric function and upon Boldyreff's (16) belief, supported by Carlson (17), Elman (18) and many others, that a high and constant gastric acidity is reduced by some neutralizing mechanism, probably a reflux of alkaline pancreatic juice which depends on normal pyloric activity. This belief was developed further by Olch (19) who looked upon the prepylorus, the pylorus and the first part of the duodenum as a single organ the function of which is that of a mixing chamber in which acid gastric contents are rendered innocuous to duodenal mucosa.

In spite of many conflicting observations, attention is directed again and again to a disturbance of normal regurgitation as one of the most important links in the chain leading to clinical peptic ulceration. There are many possibilities for central, reflex and local origins of pylorospasm which breaks up the normal mechanism with resulting faulty regurgitation leading to hyperacidity and, possibly in the case of erosions, continued ulceration. It is a well known fact that either pyloroplasty or gastroenterostomy can successfully overcome pylorospasm: pyloroplasty by entirely breaking the pyloric ring, gastroenterostomy by creating an emergency exit and means of regurgitation and abolishing spasm.

Inasmuch as pylorospasm seems to be so important in this disorder it was inevitable that the part played by the vegetative nervous system should be closely scrutinized. Furthermore its influence on the blood vessels in the usual region of ulceration should not be ignored.

Dating at least from Cammerer (20) in 1828, there have been innumerable reports of experiments involving the vagus or the splanchnic nerves and many conclusions have been contradictory. Apparently ulcers have been produced by experimentally disturbing the equilibrium of the vegetative nervous system by direct attack on either the vagus or the splanchnic nerves. In general the vagus stimulates activity while the splanchnic nerves inhibit, and the weight of evidence supports Pavlov (21) in his belief that the vagus is the main pathway

between the brain and the upper gastrointestinal tract. Greggio (22) and later Hughson (23) noted that vagus neurotomy brings about delayed motility and hypotonus of the pylorus which allows increased patency of the pyloric lumen. Beaver and Mann (24) found that in animals in which surgical duodenal drainage had been performed, ulcers developed as usual when the splanchnic nerves had been cut but did not occur invariably after section of the vagus nerves. Durante (25), as well as Dalla Vedova (26), found ulceration after cutting the splanchnic nerves in otherwise intact dogs. Cushing (27) suggested that hypothalamic disturbances gave rise to the stimuli which affect the activity of the gastrointestinal tract by way of the vagus nerves. Clinical application of the idea that section of these nerves might logically be tried in patients with peptic ulcer had been made by a few observers but it is Dragstedt (28) who is responsible for the present great interest in its use. His results and the results of others working along similar lines when put to the test of the sufficiently long time interval necessary in dealing with peptic ulcer may shed further light on the problem.

The following is a brief summary of one of the efforts made during the period 1920 to 1930 to throw further light on the resistance of gastric and duodenal mucosa to the action gastric juice following extensive surgical alteration of gastrointestinal continuity. These particular observations were not reported at the time (1925) because of the uncertainty regarding any inferences to be drawn from them. However, later consideration warrants the belief that this very uncertainty may in itself justify comment. The hitherto unpublished drawings by Max Brödel are further stimulus to publication.

1. *Implantation of opened segments of intestine into the stomach wall.* This was done in nine dogs. A segment about 10 cm. long taken from the jejunum or upper ileum, attached to its mesentery, was opened and implanted into a defect made just above the greater curvature in the lower fundus. Intestinal continuity was then restored.<sup>1</sup> The intestinal mucosa showed no ulceration in its foreign environment, even in

<sup>1</sup> This necessitated the use of a rapid means of intestinal anastomosis and presented the opportunity to try several different methods. One of the most satisfactory was based on the Parker-Kerr technique. Very thin-bladed clamps made for the purpose were used and a single Cushing continuous mattress suture of 000 chromic catgut, interrupted in three places, completed the anastomosis. Plate 4 illustrates a healed anastomosis with the suture line scarcely visible.

those few instances where silk sutures had penetrated the lumen. In two dogs the patch had puckered into a pouch which contained stones, bones, etc. In these there were superficial erosions which did not penetrate beyond the mucosa (Figure 1 and Plates 1, 2 and 3).

This procedure was first used by Katzenstein (29) and has been repeated by many others (11, 14). When ulcers have been found it was suggested they may be due entirely to nutritional disturbances resulting from inadequate circulation (Hotz) (30). This may account for the ulcers encountered by Morton (15) and de Takats and Mann (31) in patches implanted on the lesser curvature. In this position the

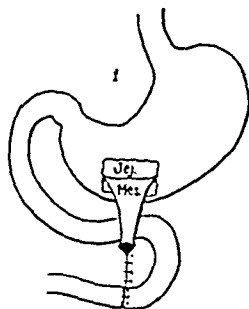


FIG. 1.

blood supply through the mesenteric pedicle may well be impaired by pressure of the animal's stomach. Morton (15) carried this procedure a step further and found ulceration in the patches when Mann's surgical duodenal drainage was also done. It is interesting to note that Andrus, Lord and Stefko (32) found a diminution of gastric acidity following patch implants and used this procedure in the treatment of clinical peptic ulcer.

2. *Stomach flaps reimplanted into gastric-wall.* This was done to compare the healing with intestinal implants. Large flaps were made and the pedicle narrowed down practically to a single artery and vein. Not until these vessels were of no appreciable size was there any evidence of susceptibility of the gastric mucosa to erosion or ulceration. Figure 2.

3. *Attempts to convert the stomach and first part of the duodenum into a single pouch.* Figures 3, 4 and 5 illustrate the methods used. Only

one dog in which the operation shown in Figure 3 was done lived as long as ten days. That dog was killed in a dog fight. The mucosa was intact. Four other dogs died within three days of rupture or perforation near the blind end of the duodenum.

The procedure shown in Figure 4 was done in two dogs. One died on the fourth day with no cause evident at autopsy. The other dog

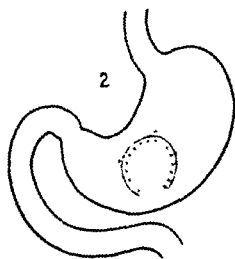


FIG. 2.

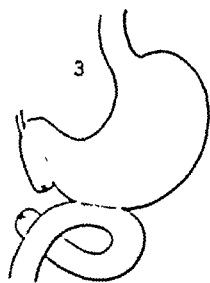


FIG. 3.

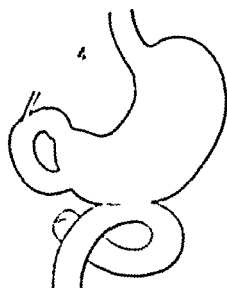


FIG. 4.

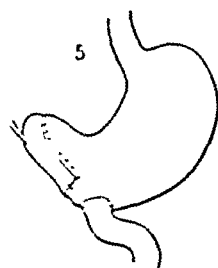


FIG. 5.

died after three weeks of mange and inanition. The mucosa was everywhere intact.

Figure 5 illustrates the operation done on three dogs. Two dogs died on the third day of perforation or rupture of the duodenal flap. The third dog died on the fourth day of no discernable cause and in this dog the mucosa was intact.

4. *Diversion of bile to mid-ileum by cholecyst-ileostomy and division of common duct. Centering of stomach and descending duodenum into a single pouch by a long Finney pyloroplasty.* Three dogs were used. One

dog died during the first 2 hours. Two dogs remained in good condition 8 and 10 weeks respectively. At autopsy the mucosa was everywhere intact (Figure 6).

5. *Modified Exalto (Mann-Williamson) procedure.* Surgical duodenal drainage by pylorectomy, drainage of descending portion of duodenum into mid-ileum and anastomosis between stomach and remaining duodenum (Figures 7, 8 and 9).

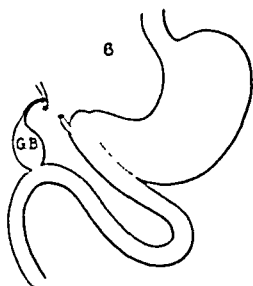


FIG. 6.

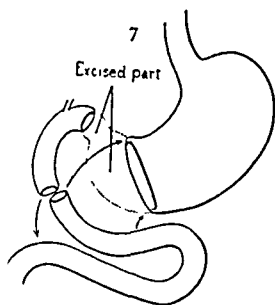


FIG. 7.

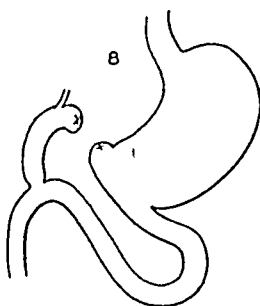


FIG. 8.

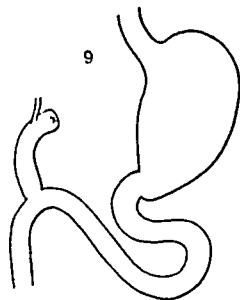


FIG. 9.

This procedure, with variations has been used by many investigators. Four dogs survived this operation for at least 24 hours but three in which end-to-side anastomosis was done died before the fourth day because of perforations in the duodenum near the suture line. One dog, end-to-end anastomosis, died on the 28th day of inanition. The mucosa was intact.

6. *The Chlumsky (33) procedure.* Pylorectomy, with drainage of duo-

*denal contents into upper portion of stomach. Side-to-end gastrojejunostomy* (Figure 10). This operation was done on three dogs. All survived for at least two months, and all showed chronic ulcers in the afferent loop of jejunum (Plate 5). One dog was sacrificed after three months during which time the interior of the stomach had been observed at intervals gastroscopically. By this means the retarded healing of the lower gastro-jejunal anastomosis was seen.

This surgical arrangement seems to be the antithesis of the Exalto procedure since duodenal contents are drained toward rather than away from the stomach. Ulcers follow the use of either procedure but in my

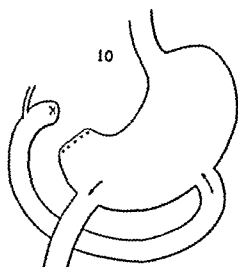


FIG. 10.

hands at least, the Chlumsky procedure gave rise to chronic ulcers as contrasted to the acute perforating ulcers of the Exalto procedure, although Mann and Williamson found chronic ulcers in a high percentage of their animals. This operation was tried by Kelling (34) and later by McCann (35) with similar results. McCann found jejunal ulcers in 80% of his animals and emphasized the importance of the mechanical factors involved, as had Mann and Williamson. This point of view was not shared by Steinberg and Proffitt (36).

The inferences to be drawn from observations following procedures such as these are at best not clear and they are frequently contradictory. It would appear that gastric mucosa is resistant to injury as a result of cutting down but not completely blocking its circulation. Intestinal mucosa with intact circulation is likewise resistant to the effect of a gastric environment when implanted as a patch in a defect of the stomach wall. Morton found this to be a relative resistance and affected by concomitant surgical duodenal drainage. The absence of



bile from the upper duodenum is not conducive to duodenal ulceration in the presence of a wide opening between the stomach and duodenum. Others, notably Grey (37) and Elman (38) have shown that the absence of pancreatic juice is of importance. Surgical duodenal drainage to the lower ileum is as shown by Exalto, Mann and Williamson, and others, a procedure which is followed by a high incidence of ulcers in that portion of the intestine used to restore gastrointestinal continuity. This might well imply that the absence of upper duodenal secretions is the factor which renders intestinal mucosa sensitive to the effect of gastric juice were it not for the fact that complete backward diversion into the stomach (the Chlumsky procedure) is also followed by ulceration.

There are apparently factors other than simple mechanical neutralization which are involved in the production of peptic ulcer by these extensive surgical procedures. Such procedures are usually followed by marked disturbances in the dog's nutrition and it is well known that ulceration is common in cachectic dogs. This comment may well be directed toward other extensive experimental surgical procedures not included in this discussion. Perhaps the most direct conclusion to be drawn from these and other similar reported experiments dealing with the part played by digestion in the production of chronic peptic ulcers is that the mechanism which makes digestion locally effective has not been fully explained.

#### BIBLIOGRAPHY

1. CRUVEILHIER, J.: *Anatomic pathologique du corps humain*. Paris, 1829-35, 1. livr. X, XX; 1835-42, 2 livr. XXX, XXXI.
2. ROKITANSKY, C.: *Ueber das perforirende Magengeschwür*. *Med. Jahrb. d. k. k. öster Staates*, Wien, 27 (n. f. 18): 184, 1839. *A manual of Pathological Anatomy*. London. Sydenham Soc. 2: 31, 1849.
3. COHNHEIM, J.: *Vorlesungen über allgemeine Pathologie*. 2: 52. 1880. *Lectures in General Pathology*. London. The New Sydenham Soc. 3: 875, 1890.
4. WELCH, W.: *Simple Ulcer of the Stomach*. *Pepper's System of Medicine*, 2: 480, 1885.
5. IVY, A. C.: *Contributions to the physiology of the stomach*. LII. *Studies on gastric Ulcer*. *Arch. Int. Med.*, 25: 6, 1920.
6. MACCALLUM, W. G.: *On the pathogenesis of chronic gastric ulcer*. *Amer. Med.* 8: 452, 1904.
7. BERG, B. N.: *Gastric ulcers produced experimentally by vascular ligation*. *Arch. Surg.* 54: 58, 1947.

8. EXALTO, J.: *Ulcus jejuni nach Gastroenterostomie. Mitt. a. d. Grenzgeb. d. Med. a. Chir.*, **23**: 13, 1911.
9. MANN, F. C.: The chemical and mechanical factors in experimentally produced peptic ulcer. *Surg. Clin. N. Amer.* **5**: 753, 1925.
10. MANN, F. C. AND WILLIAMSON, C. S.: The experimental production of peptic ulcer. *Ann. Surg.* **77**: 409, 1923.
11. MANN, F. C.: The effect on the jejunal mucosa of exposure to gastric juice. **35**: 289, 1917.
12. MANN, F. C.: Production and healing of peptic ulcer. An experimental study. *Minn. Med.* **8**: 638, 1925.
13. MORTON, C. B.: Observations on peptic ulcer. I. A method of producing chronic gastric ulcer. A consideration of etiology. *Ann. Surg.* **85**: 207, 1927.
14. MANN, F. C.: IV. Patch transplants of jejunum to stomach. *Ann. Surg.* **85**: 879, 1927.
15. MANN, F. C.: V. Findings in experimentally produced peptic ulcer: etiologic and therapeutic considerations. *Ann. Surg.* **87**: 401, 1927.
16. BOLDYREFF, W.: The self-regulation of the acidity of the gastric contents. *Quarterly Journ. Exp. Physiol.* **8**: 1, 1914.
17. CARLSON, A. J.: The secretion of the gastric juice in health and disease. *Physiol. Rev.* **3**: 1, 1923.
18. ELMAN, R. AND ROWLETTE, A. P.: The role of the pyloric sphincter in the behavior of gastric acidity. *Arch. Surg.* **22**: 426, 1931.
19. OLCH, I. Y.: Duodenal regurgitation as a factor in the neutralization of gastric acidity. *Arch. Surg.* **16**: 125, 1928.
20. CAMMERER, quoted by ROST, F.: *Pathological Physiology*. Phila. 1923.
21. PAVLOV, I. P.: *Die Arbeit der Verdauungsdrüsen*. Wiesbaden 1898.
22. GREGGIO, E.: Des Ulceres gastro-duodénaux. *Arch. de Méd. exper. et d'anat. path.* **27**: 533-590, 1916-17.
23. HUGHSON, W.: Effect of vagus neurotomy on the pyloric sphincter. *Journ. Amer. Med. Assn.* **88**: 1072, 1927.
24. BEAVER, M. G. AND MANN, F. C.: The production of peptic ulcer after section of the gastric nerve. *Ann. Surg.* **94**, 1116, 1931.
25. DURANTE, L.: The trophic element in the origin of gastric ulcer. *Surg., Gyn. and Obst.* **22**, 399, 1916.
26. DALLAVEDOVA, R.: Experimenteller Beitrag zur Kenntnis der Pathogenese des *Ulcus ventriculi*. *Arch. f. Verdauungskr* **8**: 3, 2544, 411, 1902.
27. CUSHING, H.: Peptic ulcers and the interbrain. *Surg. Gyn. and Obst.* **55**, 1, 1932.
28. DRAGSTEDT, L. R.: Vagotomy for gastro-duodenal ulcer. *Ann. Surg.* **122**, 973, 1945.
29. KATZENSTEIN, M.: Der Schutz des Magens gegen die selbst Verdauung nebst einem Vorschlag zur Behandlung des *Ulcus Ventriculi*. *Berl. Klin. Wchnschr.* **34**, 1749, 1908.

30. HOTZ, G.: Versuche über die Selbstverdauung des Darms in Magen. Mitt. a. d. Grenzgeb. 21, 143, 1090.
31. DETAKATS, G. AND MANN, F. C.: The effect on the jejunal mucosa of transplantation to the lesser curvature of the stomach. Ann. Surg. 85: 698, 1927.
32. ANDRUS, W. D., LORD, J. W. AND STEFKO, P.: Effects of pedicle grafts of jejunum in the wall of the stomach on gastric secretion. Ann. Surg. 118: 499, 1943.
33. CHLUMSKY, V.: Über die Gastro-Enterostomie. Bruns Beitrage 20: 2313, 1898 and 27, 311, 1900.
34. KELLING, G.: Studien zur Chirurgie des Magens. Arch. f. Klin. Chir. 62: 307, 1900.
35. McCANN, J. C.: Experimental peptic ulcer. Arch. Surg. 14: 600, 1929.
36. STEINBERG, M. E. AND PROFFITT, J. C.: The etiology of post-operative peptic ulcers. Arch. Surg. 25: 819, 1932.
37. GREY, E. G.: The Diversion of the pancreatic juice from the duodenum into the stomach. Its effects upon the level of gastric acidity and upon the pancreas. Journ. Exper. Med. 26: 825, 1917.
38. ELMAN, R.: Probable influence of the pancreatic juice in the regulation of gastric acidity. Arch. Surg. 16: 1256, 1928.

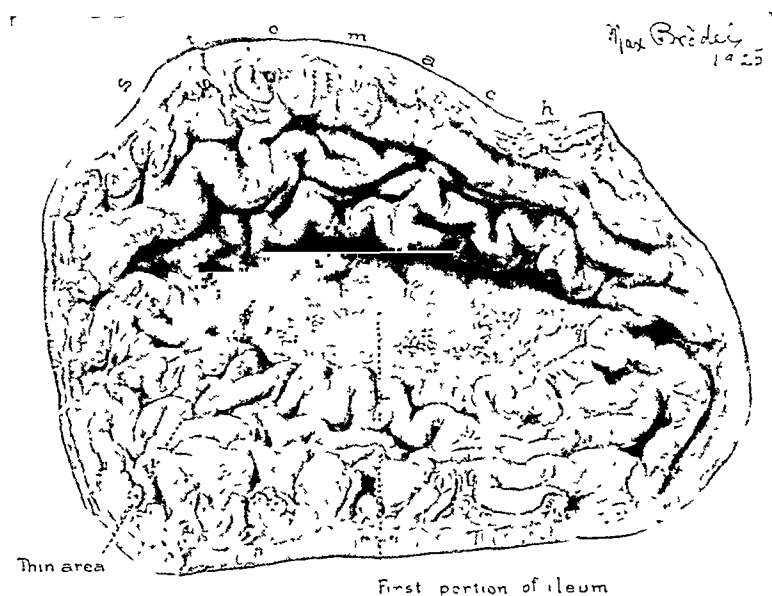


PLATE 1. ANTERIOR WALL OF STOMACH, POST-MORTEM FORMALIN FIXATION  
The "thin area" proved on microscopic examination to be a mucosal erosion.

PLATE 2. THREE INTERRUPTED SILK SUTURES HAVE PENETRATED THE LUMEN,  
WITH NO RESULTING ULCERATION.

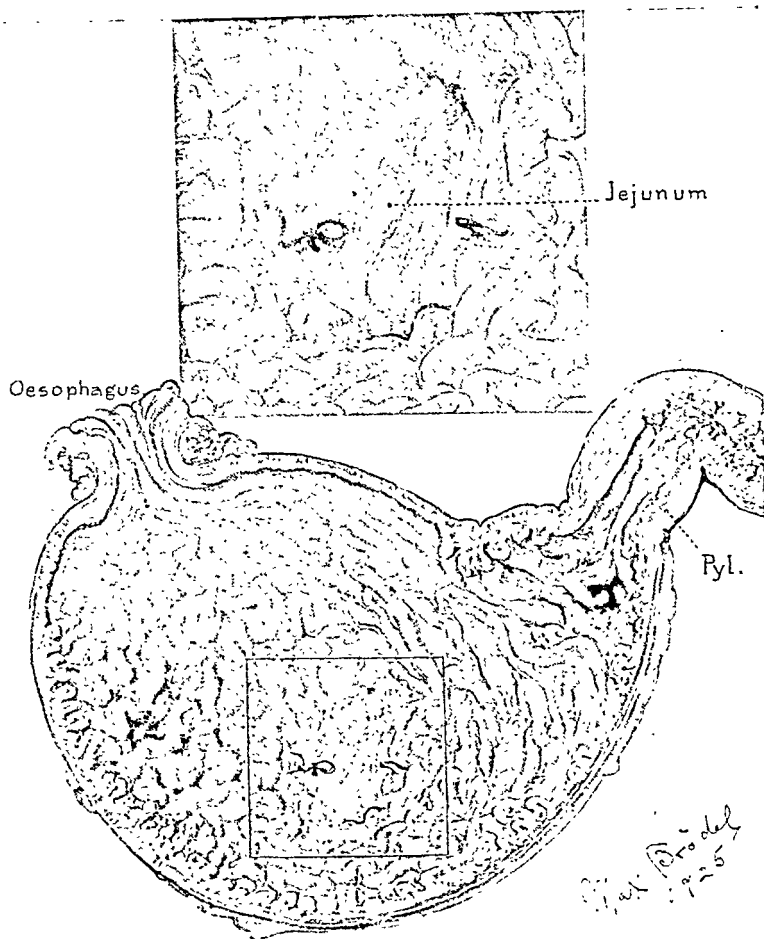


PLATE 3. GASTROSCOPIC EXAMINATION OF IHL INTESTINAL PATCHES WERE  
MADE AT FREQUENT INTERVALS BY DR. EDWIN N. BROYLES

The intact mucosa was clearly visible.

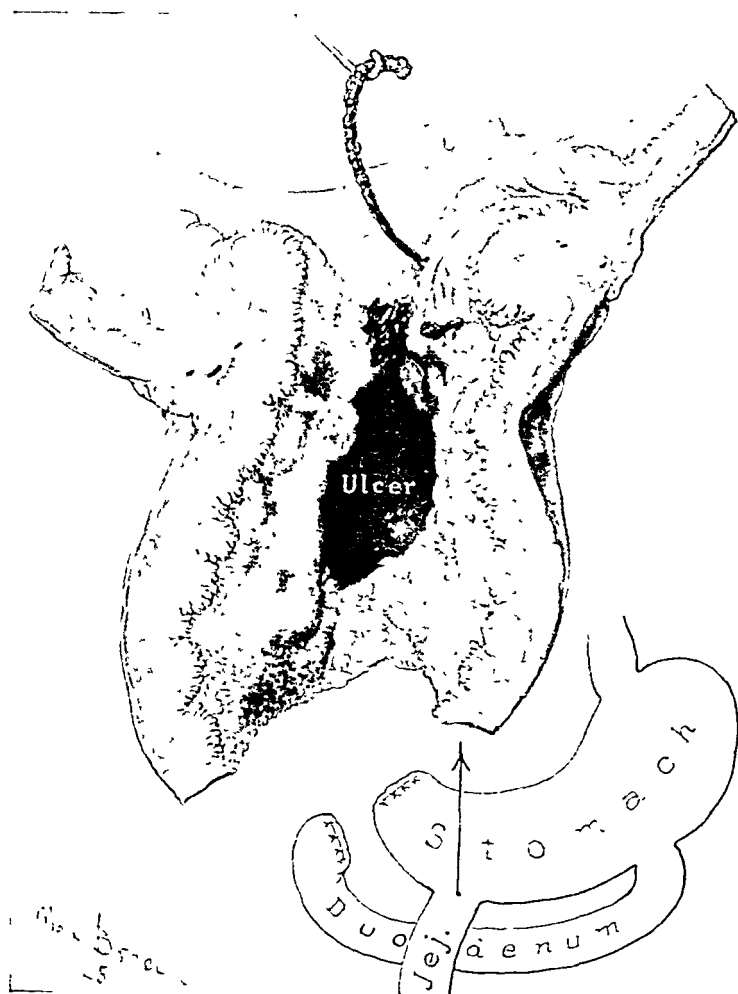




PLATE 4. HALALD END-TO-END INTESINAL ANASTOMOSIS PARKER-KERR  
TECHNIQUE.



PLATE 5. ULCER IN AFFERENT LOOP OF JEJUNUM FOLLOWING CHLUMSKY  
PROCEDURE





## PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

HELD IN HURD MEMORIAL HALL, MONDAY, MARCH 10, 1947

*Diphtheritic Polyneuritis.* DR. GEORGE D. GAMMON (Department of Neurology, University of Pennsylvania).

Shortly after the end of the European war the author had the opportunity of studying a group of cases of multiple peripheral neuritis among American, British and German troops in the Mediterranean Theatre. The largest number examined (75) were among German personnel. The cases could be classified on clinical grounds into postdiphtheritic palsy, postinfectious neuritis, sulfonamide neuritis, serum neuritis, and a group not readily identified as to cause. The preponderance of cases was due to diphtheria. After the clinical classification was made, cultures of nose and throat for diphtheria were made and blood drawn for antitoxin level determination as described by Dr. Schoenbach.

The clinical picture of diphtheritic neuritis can be readily recognized in its typical form. After nasopharyngeal infection, palatal palsy is followed by paralysis of accommodation and by the slow development of tingling and weakness of the limbs. Although motor weakness and reflex loss may exceed the sensory impairment, blunting or actual loss of cutaneous and deep sensation occurs.

The slow time course of the process is a characteristic feature which clearly distinguishes diphtheritic neuritis from the sulfonamide and the serum group, as well as some of the cases described by Guillain.

A few cases of skin diphtheria were studied, but none were seen at the time of open ulcers. The prevalence of carriers raises the question of secondary infection of skin lesions rather than primary involvement.

*Laboratory Studies on Diphtheritic Polyneuritis.* DR. EMANUEL B. SCHOENBACH (Department of Preventative Medicine, School of Hygiene).

Many cases of polyneuritis had been observed among the Armed Forces in Italy during 1944 and 1945. Because of a high spinal fluid protein unassociated with an increase in cells, these neuritic cases were classified as Guillain-Barré syndrome.

Review of many of the case histories suggested diphtheritic infection as the likely etiologic cause for the polyneuritis. During the summer of 1945 seventy cases of peripheral neuritis in one area were studied. A detailed history and comprehensive neurological examination of each case was undertaken and all available laboratory data was reviewed. It was found that the cases could be subdivided clinically into characteristic groups. They were:

- 1) Typical postdiphtheritic neuritis (42)
- 2) Sulfonamide neuritis (methyl sulfathiazole, Uleron) (13)
- 3) Postinfectious neuritis with severe general sepsis (6)
- 4) Unclassified atypical neuritis (9)

Laboratory investigation consisted of nose and throat cultures for *C. diphtheriae* on two successive days, employing several types of media for isolation, and quantitative titrations of the serum antitoxin levels. These laboratory studies were extended for control purposes to include 112 individuals without recent history of sore throat or polyneuritis and 17 cases of known convalescent pharyngeal diphtheria.

The results of the laboratory study may be summarized as follows:

1. Virulent *C. diphtheriae* was isolated from 52.6% of those convalescent from acute diphtheria (4-8 weeks after onset); from 17.0% of the normal control group, and 12.0% of those classified as diphtheritic polyneuritis.

2. The antitoxin content of the serum was lower among the postdiphtheritic polyneuritic and convalescent diphtheria groups than among the control group. 50% of the former groups had levels below 0.01 units of antitoxin as compared to 22% among the control asymptomatic group. This difference was essentially unchanged when less than .0025 units of antitoxin was employed as a basis for comparison.

3. Examination of the strains of *C. diphtheriae* for mitis, gravis, intermedius, etc. revealed no distinctive type characteristic which could be employed to distinguish *C. diphtheriae* isolated from cases of diphtheria, polyneuritic cases or the healthy control group.

The cases of diphtheritic polyneuritis were distinctive on clinical grounds. Laboratory confirmation of undiagnosed diphtheritic infection preceding the onset of the polyneuritis was unsuccessful. The high incidence of diphtheritic infection in the community, the relatively small number who manifest overt clinical disease, the late onset of postdiphtheritic polyneuritis, and the poor antibody response following the disease make such retrospective laboratory diagnosis difficult in diphtheria.

*Dr. J. E. Howard:* It is somewhat encouraging to the doctor to find that the specialists have difficulty in interpreting the etiological agent when they are confronted with patients with peripheral neuritis. This is a pretty broad subject and we have with us tonight a very distinguished visitor. I would like first to ask his comments on the subject of tonight's presentation—Sir Charles Symonds.

*Sir Charles Symonds:* I have listened with interest to the contributions that have been given tonight. Dr. Gammon has presented us with a very clear and convincing picture of the clinical aspects of diphtheritic polyneuritis. I am sure he is right and that the clinical picture is one that, as a rule, deserves a positive diagnosis of its own right, even if there is no positive test available. When there occurs the typical sequence of palatal paralysis and then, after delay, the polyneuritis is present, there can be little doubt about the typical clinical diagnosis, but, as he has pointed out to us, the palatal paralysis and ciliary paralysis may occasionally be absent and then we are faced with a polyneuritis which hasn't got a whole lot of diphtheritic polyneuritic characteristics. Now, we have got to note

also that the delay between the time of the infection—the time the culture is positive—and the development of the polyneuritis may be quite a long one. In these cases I noticed one in which the interval was of twelve weeks. Intervals longer than that have been recorded. In one of the cases seen in Palestine during the recent war, the interval between positive culture and the first symptoms of polyneuritis was 149 days. Now, this carries to the clinical neurologist a real lesson. When he meets with a case of polyneuritis of obscure etiology he should always think of the possibility of a diphtheritic polyneuritis, 'even if the history of palatal and ciliary paralysis is not forthcoming, and look for the history of sore throat or possibly cutaneous sore away back in the story, even as long as three or four months.

In regard to the Guillain-Barré syndrome—Guillain and Barré thirty-one years ago made an important contribution to knowledge when they observed that in certain cases of polyneuritis there might be a high protein in the spinal fluid without any pleocytosis. It so happened that the group of cases which engaged their attention was one with particular clinical features. During the first World War in France in 1916 and 1917 there was quite an outcrop of cases which were described by Holmes independently in 1917 under the title of acute infective polyneuritis. Holmes gave a very vivid and clear picture of that disease, with its bilateral facial palsy, rather marked developments, severe involvement of the proximate muscles and relative escape of sensory nerve fibers. He, too, observed the increase of protein in the cerebrospinal fluid. But, as Dr. Gammon has remarked, and others before him, we have since learned that in many other varieties of polyneuritis this same picture may be found in the cerebrospinal fluid. In fact, the syndrome described by Guillain-Barré is not specific for the disease in which they were interested at that time. Further than that, in the disease which was described by Holmes, and in which Guillain-Barré found this picture in the cerebrospinal fluid, we know now that, in addition to the high protein, there may be a high cell count. Dr. Gammon gave us a quite typical example of acute infective polyneuritis in which the protein content was 150 milligrams and in which there was also a cellular increase. So I think it is about time that we cease to think in terms of the Guillain-Barré syndrome. I think it is an excellent piece of scaffolding which we should now be prepared to tear down when the building is a little more completed. The tendency to think in terms of a disease, and to think that that disease was discovered by Guillain-Barré, and that it comprised an acute infective polyneuritis with a specific type of cerebrospinal fluid which isn't encountered in other types of polyneuritis, is quite wrong. I think it would be better if we just recognized the direct characteristics of that polyneuritis and that in any of them, including diphtheritic, we may find this curious syndrome in the spinal fluid.

*Dr. H. M. Thomas:* Dr. Howard, I felt as I listened to the papers that perhaps I would be pressed into service tonight under somewhat false pretenses because I really saw very little of the disease that has been discussed tonight, diphtheritic polyneuritis. I think perhaps Dr. Harvey saw the cases that we had in the Pacific



more concentratedly than anyone else in the general hospital where a group of cases were studied representing various forms of neuritides and the diagnosis of diphtheria was considered in each one. I believe that possibly he would be willing to tell us about that relatively small group.

I was interested in another aspect of the talks tonight. In contemplating the diagnosis of diphtheritic polyneuritis, it seems important that diphtheria should be prevalent in the community. When I went out to the Southwest Pacific I stopped on the way in the Central Pacific and in the South Pacific, and in each place was told of the fact that they were finding cutaneous diphtheria, and that they couldn't find it merely by the ordinary culture methods on Löffler's Media, but special cultures and procedures were necessary, so we were forewarned, and all through New Guinea we looked for cutaneous diphtheria. We didn't find any until suddenly, almost simultaneously, at Hollandia and Finschhafen, there were three outbreaks of clinical pharyngeal diphtheria. These were carefully studied and two of them were found to have been connected with the skin ward of the hospitals where they broke out. In culturing the cases—all the cases on the skin ward in those hospitals—it was found that a number of them were suffering from cutaneous diphtheria, not only in ulcers but in other eczema-like types of skin lesions. In retrospect, however, following these outbreaks, it seemed to us fairly clear that we hadn't been seeing any diphtheria before because there had been no recognized pharyngeal diphtheria. I think a very few cases of peripheral neuritides eventuated from these outbreaks. The picture changed in the Philippines and there, suddenly, an outbreak of virulent poliomyelitis developed and four out of five first cases died in November of 1944. The fear was that we were encountering Japanese B encephalitis, or some other form of very virulent virus disease. The study of that is another rather long story and it only can be mentioned here to say that there were two groups fighting on the island of Leyte, the group around Tacloban and Palo, and the group farther south, and there were two different forces that came into these two areas. One came up from New Guinea and the other had come in from the Central Pacific from Hawaii. Simultaneously poliomyelitis broke out in these two areas. These outbreaks were very different because of the first seventeen cases in November and December in the Tacloban area eleven died, whereas of a comparable nineteen cases in the Dulag area only one died. In reviewing the cell count of the spinal fluid it was found that in the virulent group no case had fewer than 500 cells in the spinal fluid, and many had over 30,000 cells; whereas in the other group many had cell counts under 50 and around 20, 16, 7, 8, 4, etc. The one case that died in the Dulag area had 347 cells on the first count. It seemed that we were seeing two outbreaks almost identical clinically in that there was rapid paralysis often of an ascending variety. Subsequent review, however, indicated that the troops came from different staging areas and brought with them different diseases, namely poliomyelitis and Guillan-Barré syndrome, which simultaneously produced epidemics in the Leyte Island campaign. These cases differed from the diphtheritic group in that they came in to the hospital

complaining of severe headache and pain, and very promptly within the next few days, or a week, became seriously ill or died of an ascending form of paralysis with a bulbar lesion. No clear clinical differentiation was made at the time and battle conditions made it impossible to study thoroughly the spinal fluid albumin content. There were some sensory disturbances. Some of the patients went back to general hospitals in New Guinea and were studied later and found to have high spinal fluid proteins long afterwards, but so also had the late poliomyelitis cases. I think our experience then was not comparable to that of the Mediterranean group, but was also extremely interesting in that many forms of neuritides do occur. The bedside differential diagnosis is quite difficult, as has already been said, and these various clinical pictures should be borne in mind.

*Dr. Howard:* Dr. Baker, would you care to make a few remarks?

*Dr. B. M. Baker:* I listened with great admiration to the reports of the comprehensive studies upon neuritis made in the Mediterranean Theatre and I am sorry that I have no report of comparable importance to make on experiences with the Armed Forces in the Pacific. Dr. Thomas has indicated that in the Pacific we very soon began to see patients with peripheral neuritis of obscure etiology. The first progress was made when it was recognized that a good many cases of peripheral neuritis developed following unsuspected pharyngeal diphtheria.

There was nothing very unusual about the type of neuritis we encountered. I will confine my remarks largely to the importance of skin diphtheria as a dangerous source of infection.

Many soldiers who were evacuated from forward areas to hospitals in the rear had a wide variety of skin lesions. These lesions frequently had the typical characteristics of tropical ulcers or desert sores. Bacteriological investigation soon emphasized the importance of skin diphtheria. The importance of cutaneous diphtheria has been previously recognized both in civilian practice and in the military forces. Walsh pointed out the relationship between cutaneous diphtheria and neuritis.

In our experience careful studies were made upon 700 odd cases of tropical ulcers in a rear area where there were adequate facilities for thorough bacteriological investigation including virulence tests. One hundred ninety of these 700 cases of tropical ulcers were found to harbor toxigenic diphtheria. From these 190 cases 13 cases of peripheral neuritis were detected. It is worth mentioning that the latent period between the onset of skin infection and neuritis in these cases varied from three to six months, in contrast to the shorter period in which neuritis follows pharyngeal diphtheria. The neuritis was usually confined to the extremities. In general it was mild. Interest centered largely in epidemiological considerations and a number of studies upon natives were carried out. There was a high incidence of cutaneous diphtheria in the natives, and secondary infection of yaws and other common skin lesions constituted the chief hazard. There was a surprisingly low incidence of diphtheria susceptibility in adult natives in whom nasopharyngeal

diphtheria is apparently very uncommon. This finding emphasized the probable importance of skin infections among natives as the source from which our troops derived this infection.

*Dr. M. C. Pincoffs:* Dr. Howard, I have very little to contribute to this discussion as far as experience in the field with diphtheria is concerned. I am mainly filled with regret that in the theatre in which I stayed for some time we undertook nothing in the way of such a careful and valuable study as that which has been presented to us this evening. I found it most instructive and helpful in clarifying some ideas on this subject.

The demonstrated frequency of albumino-cytological dissociation in the spinal fluid of patients with diphtheritic infection naturally raises the question as to whether the clinical cases described by Guillain-Barré were likewise of diphtheritic etiology.

Cases seen in civilian life prior to the war usually had somewhat different clinical features from those described by Dr. Gammon this evening. The onset was frequently abrupt, the spread of muscular weakness rapid, facial diplegia common and fatal involvement of the respiratory musculature not infrequent. The spinal fluid characteristically showed no increase of cells and marked increase of protein. Diphtheria was not suspected in these cases. In view of the variance of clinical features it seems quite probable that diphtheritic infection may be only one of several etiologic agents which may produce central nervous system lesions accompanied by the changes in the spinal fluid described by Guillain-Barré.

*Dr. Howard:* Are there any further comments or questions?

*Dr. P. H. Long:* Yes, I would like to make a comment, Dr. Howard. These two reports have interested me very much because about two years ago this time I was burning up the wires trying to get some people over to study this problem in Italy; while it was not a pressing problem it was an extremely interesting one. Twenty-two months ago Dr. Gammon and Dr. Schoenbach arrived. Tonight I have heard their reports for the first time. We asked them over to study these diseases for us and I find they had to study them in the camp of the enemy, which is very much like the Army. But to become serious—the history of polyneuritis in the North African and Mediterranean Theater is rather interesting because the first time that it came to our attention was in January 1943 when we had an outbreak of eight or nine cases of a rapidly ascending paralysis in troops in the Casablanca area. Most of these men died rather promptly, and because of the fact that there were only evacuation hospitals in that area the bacteriological studies were not very complete. There was relatively little recognized clinical diphtheria in M.N.T.O. and M.T.O. Recognition was good in the North African and Mediterranean Theater. We had very little recognized diphtheria through 1943 and not until January 1944 did we get much diphtheria. This isn't so difficult to under-

stand when you realize that our troop strength up to that time was comprised of regular Army divisions who had been mobilized and in training since 1940. They had trained all over the United States and we probably had a fairly high percentage of immune troops among them. Late in 1943 we began getting in our terrific drafts of replacements, many of whom were boys who had been in the Army only eight or nine weeks and had only been away from their homes for about that length of time. Through 1944 we began to get a rise in clinical diphtheria and the problem of infectious polyneuritis first arose as a diagnostic problem in late December 1943 and in January 1944. I had seen a fair amount of diphtheritic polyneuritis in the British hospitals which followed clinical nasopharyngeal or pharyngeal diphtheria, but the thing which characterized many of our early cases of polyneuritis and which puzzled me were the people who did not have a history of clinical diphtheria, and in whose cases one could go back to his surgeon who remembered that the patient's throat was a little red ten weeks before. These people did not have what you and I would call clinical diphtheria, but eight or ten weeks later developed a type of polyneuritis. In the summer of 1944 in American and British hospitals there were instances of a rapidly progressing generalized polyneuritis which involved the face, with a spinal fluid protein of 115 to sometimes 800 milligrams per cent and which would clear up with great rapidity. I am quite sure it wasn't poliomyelitis, but at the present time I am not sure what it was. The disease fitted the description of the Guillain-Barré syndrome. Unfortunately there was not time for Doctors Gammon and Schoenbach to study them because when they came over redeployment was being carried out, and they were sending home everyone from our hospitals who could go on two legs. Thus they did not see most of the cases which we had hoped they would see because many of them, there, in January 1945 had been sent home and they had to study the reservoir of German cases at Merano.

There is one point I would like to end up on. I was brought up in the school of the South Department at Boston where the chief of the infectious service, Dr. Place, used to tell us to figure out how much antitoxin you should give a patient with diphtheria so as to cover his entire needs, then to give him twice as much. In the North African-Mediterranean Theater the directive read—not a request but a directive which said "*You will give*"—that a mild case of diphtheria should receive 60,000 units, a moderately severe case 150,000 units, and a severe case 250,000 units. I cannot say whether that had anything to do with decreasing the incidence of neuritis in patients with clinical diphtheria, but the fact remains that we appeared to have less post-diphtheritic neuritis than did the British Forces. Initially the British War Office required 24,000 units of antitoxin every twelve hours until 96,000 units had been given in diphtheria. I have had a great many arguments about that with Hysmann of the British Medical Service concerning this low dosage. Finally, in February 1945, the British increased their dosage of antitoxin in hopes of cutting down the incidence of post-diphtheritic neuritis.

*Dr. A. M. Harvey:* There has been a great deal of very interesting discussion on subject of polyneuritis. We saw various neurological conditions over a period of the three years in the Southwest Pacific and there, in a great number of them, along with the cases of polyneuritis, we saw many more cases in which one single peripheral nerve was involved, commonly the peroneal, more frequently the axillary, and less frequently one long thoracic nerve. At the same time we saw a much greater incidence of polyneuritis, much greater than one ever sees in a civilian population. I would like to ask Dr. Gammon and Dr. Schoenbach whether they saw any cases of that type along with the ones they examined with the multiple involvement.

*Dr. Gammon:* We saw two cases, more or less in passing, of shoulder girdle paralysis. One of them was American and the other British. I don't think we examined the American. We didn't have a chance to examine these patients, but the British patient had an atrophy of the shoulder girdle through the deltoid and supraspinatus. This was painless in his case. The man woke up one morning paralyzed in those muscles and this persisted for six months. The other man had had pain in his shoulder, but, as I said before, we never saw him. He was supposed to have a winged scapula. In the group we encountered I never saw a unilateral peroneal paralysis at all. Were the cases that you described comparable to those of Spill and Alvern and Fox?

*Dr. Harvey:* Yes, they were. We saw in the beginning very many of these cases of polyneuritis, the patients having old healed pigmented ulcers on the skin. You could not prove they had ever had diphtheria. We saw a good many of them develop under constant observation in the tropics. They had the clinical symptoms of pharyngeal diphtheria and no cutaneous lesions, and predominantly the great number showed involvement of a single nerve with no elevation in serum protein. The large majority that we saw had a fairly rapid onset and very characteristically the proximate muscles, particularly those along the shoulder girdle and pelvis, were more prominently involved. In almost no instances did any of these present any objective findings or any clinical nervous symptoms whatsoever. We saw a great many of those cases that Dr. Thomas described after the acute stage, when they had been convalescent for periods of up to four weeks. They were quite indistinguishable from Guillain-Barré syndrome of multiple paralysis with very little sensory disturbances and no marked elevation of the spinal fluid protein.

*Dr. H. A. Howe:* Could they have been poliomyelitis observed late?

*Dr. Harvey:* They probably were, but the question came up at the time as to whether or not, in a situation of that type, there may have been more than one specific type of nervous system virus active. Our knowledge is very superficial as to that type of disturbance. One group seemed to produce the clinical picture

of poliomyelitis and in the next group you might find the clinical picture associated with the Guillain-Barré syndrome. We still have a great deal to learn of peripheral neuritis and I would like to hear some discussion on it.

*Dr. Thomas:* I would like to ask Dr. Gammon a question as to whether some of the cases might have been poliomyelitis. There is now a study going on of some 52 cases that died of clinical Guillain-Barré syndrome, whatever that may be, and I am told by Major Haymaker in the Army Institute of Pathology that the pathological picture is entirely different from the picture of anterior poliomyelitis. Pathologically 2 of his 52 cases of Guillain-Barré came from our series, we did a number of autopsies also on cases that we felt sure were poliomyelitis, and they are very different pathologically. Our experience favors the concept of at least two distinct neurotropic viruses.

*Dr. Howe:* Just on the other side of the picture, I might say that recently Dr. Paul has isolated virus from the stools of some of the patients from the Philippines and Japan who had this vague syndrome. Needless to say, there was no paralysis at all upon which they ever felt it was justified to make a diagnosis of poliomyelitis. Yet the polio virus was actually isolated from the stools, which would certainly indicate that poliomyelitis was probably commoner in those theaters than generally recognized.

#### HELD IN HURD MEMORIAL HALL, MONDAY, APRIL 14, 1947

*A Report on Observations Relative to Paroxysmal Cold Hemoglobinuria.* DR. PHILIP F. WAGLEY and DR. WILLIAM H. ZINKHAM (Department of Medicine).

Five groups of experiments were carried out in the study of certain aspects of paroxysmal (cold) hemoglobinuria. The subjects were two young colored patients admitted to the hospital for treatment of syphilitic infections. Both gave histories of dark urine following exposure to cold. The Donath-Landsteiner tests were positive.

The first group of experiments dealt with the fixation of the hemolysin and complement. The second studied the effect of CO<sub>2</sub> on hemolysis under specified conditions in vitro. The third consisted of observations on the influence of carbonic anhydrase inhibitors in this hemolytic system. The fourth dealt with the possible modes of action of certain hemolytic inhibitors. The fifth group of experiments demonstrated that definite morphological changes of the erythrocytes occurred prior to hemolysis. The materials and methods employed were described.

The observations suggested that hemolysin and at least one component were absorbed in the cold by erythrocytes. In the absence of complement hemolysin was not absorbed in one case. A thermolabile fraction of complement was necessary for the warm phase of the Donath-Landsteiner reaction. Under specified

in vitro conditions  $\text{CO}_2$  seemed to effect the hemolytic system in one case and not in the other. This  $\text{CO}_2$  effect was itself subject to the influence of temperature. Sulfanilamide and cyanide inhibited the Donath-Landsteiner reaction in one case and not in the other. These substances did not destroy complement or hemolysin under the circumstances of these experiments. Apparently they did not prevent the union of the hemolysin-erythrocyte complex in the cold, but prevented the effect of such a union. If not present during this union sulfanilamide and cyanide did not prevent the hemolysis. Under specified conditions sulfanilamide and cyanide did not prevent the effect of the thermolabile component of complement in the warm phase of the Donath-Landsteiner reaction. The inhibition of these substances was reversible. During the cold phase of the Donath-Landsteiner reaction practically all the cells remained biconcave. Spherocytosis occurred during the warm phase of the Donath-Landsteiner reaction and preceded hemolysis. By employing three different methods (ocular micrometer, optical density method of Jacobs, phase difference microphotography) no definite evidence was obtained that the cell increased in volume prior to hemolysis.

It is of interest that in these two cases, presenting clinically the same disease entity, certain observations relative to  $\text{CO}_2$  and hemolytic inhibitors suggested the possibility of fundamental variants in their pathogenesis. These differences may account partially for the conflicting data reported in the literature from studies by other workers.

*Dr. Rich:* I might ask one question. The paper was very enlightening, but I would like to ask Dr. Wagley about the effects of cyanide. I didn't get that very clearly in my mind. If cyanide or sulfanilamide is added to the cells alone and then washed off will it have an effect or does it have to be present together with the hemolysin in order to have an effect?

*Dr. Wagley:* They have to be present with the hemolysin at the time of chilling.

*Dr. Harvey:* I would like to ask if there is any difference in response to antisyphilitic treatment as far as this hemolytic system is concerned.

*Dr. Wagley:* Dr. Mackenzie has had the most experience in this condition. He has stated that a study of those cases with paroxysmal hemoglobinuria who have been treated and subsequently had attacks over a long period of time did not have adequate therapy. However, there are cases in which attacks have lasted for a period of two years after antiluetic therapy. Whether there is any difference in the responses of these two types of cases to treatment cannot be answered with the present information.

*Dr. Harvey:* Has the hemolytic system been altered in any way by the treatment?

*Dr. Wagley:* It has not in its response to CO<sub>2</sub>. However, one of the cases has been out of the hospital only two weeks. What changes, if any, in the qualities of the system may ensue is not predictable.

*Dr. Howard:* Is there any further comment? If not, we will go on to the next papers which comprise a unit and will be discussed together. It will be a symposium on the experiences with vagotomy for peptic ulcer.

The symposium is published in this issue.





# PROGRESSIVE PARALYSIS OF THE NERVUS INTEROSSEUS DORSALIS: PATHOLOGICAL FINDINGS IN ONE CASE

FRANK J. OTENASEK,

*From the Department of Surgery (Neurosurgery) of the Johns Hopkins University School of Medicine*

Isolated cases of paralysis of the deep motor branch of the radial nerve (n. interosseus dorsalis) must not be very uncommon, although but few cases are recorded in the literature. Most of these have been of acute traumatic origin. Koch (1) reports such a case of section of the nerve in the supinator muscle by a stab wound, Mowell (2) after an injury by a circular saw. Occasionally a gunshot wound will, by coincidence, sever the motor and spare the sensory branches as in the cases of Marie, Meige, and Patrikios (3), and of Roussy and Branche (4). Grigoresco and Iordanesco (5) recorded a case believed to have come on after pressure on the nerve during sleep, following a sprain.

Occasionally instances of paralysis of the n. interosseus dorsalis appear to be progressive, unassociated with acute trauma and with no obvious gross lesion to account for the symptoms. An interesting account of a case possibly caused by chronic trauma was given by Guillain and Courtellement (6). Their patient, an orchestra conductor, developed weakness of extension of his fifth right finger. Ten days later extension of the fourth finger was also impaired. In six months the third finger was involved. No sensory changes appeared. Some tenderness could be elicited at the point of entrance of the n. interosseus dorsalis into the supinator muscle. It was discovered that the patient held his baton with the fourth and fifth fingers. Guillain and Courtellement ascribed the paralysis to the repeated movements of pronation and supination made while directing the orchestra.

There have been reported a few cases of isolated paralysis of the nervus interosseus dorsalis in which there was not even a history of such chronic trauma as the possible etiologic agent. Woltman and Learmonth (7) reviewed the literature on the subject and reported five cases, in one of which exploration of the nerve was carried out.

"When the supinator brevis was exposed it was found that instead of passing through the supinator the nerve passed superficially between the muscle and the aponeurosis of the common extensor. The nerve had molded a groove across the fibers of the supinator, and it seemed possible that repeated mechanical trauma imposed on the nerve by the

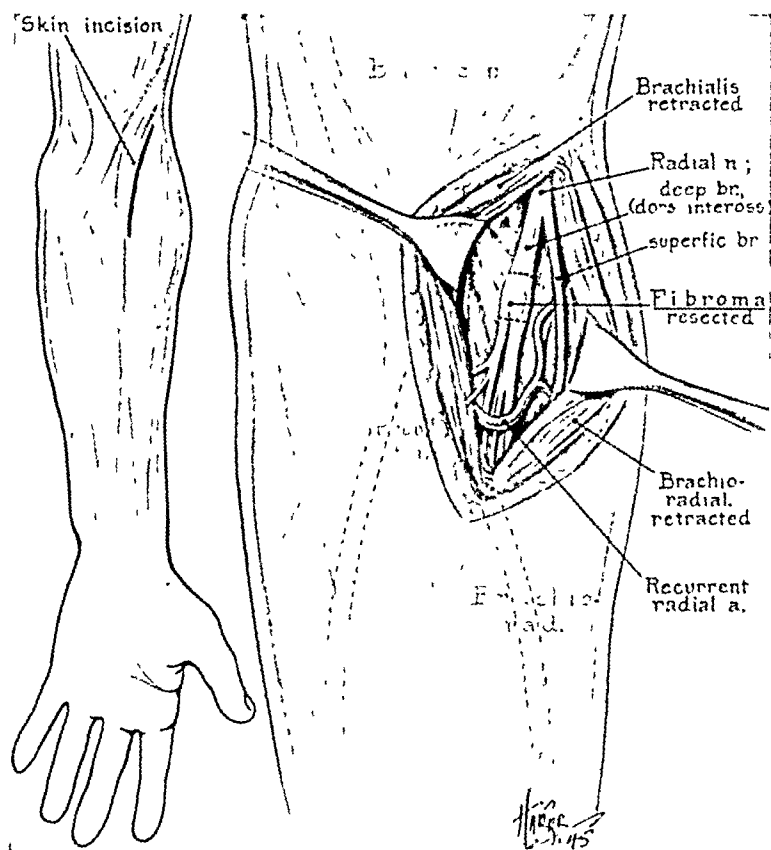


FIG. 1. OPERATIVE EXPOSURE OF TUMOR

contractions of the supinator brevis might have given rise to the neuritis." The normal course of the nervus interosseus dorsalis is through the substance of the supinator muscle.

#### REPORT OF A CASE

W. C., a white male of 21 years, complained of weakness of the left hand. At the age of 13, without history of injury of any kind, he first noted weakness of

dorsiflexion of the left thumb. This gradually worsened over a period of several years, until all fingers except the fifth were involved. He then developed weakness of extension of the wrist and finally a typical wrist drop. Examination showed extensor paralysis of the left hand and wrist, the fifth finger being partially spared.

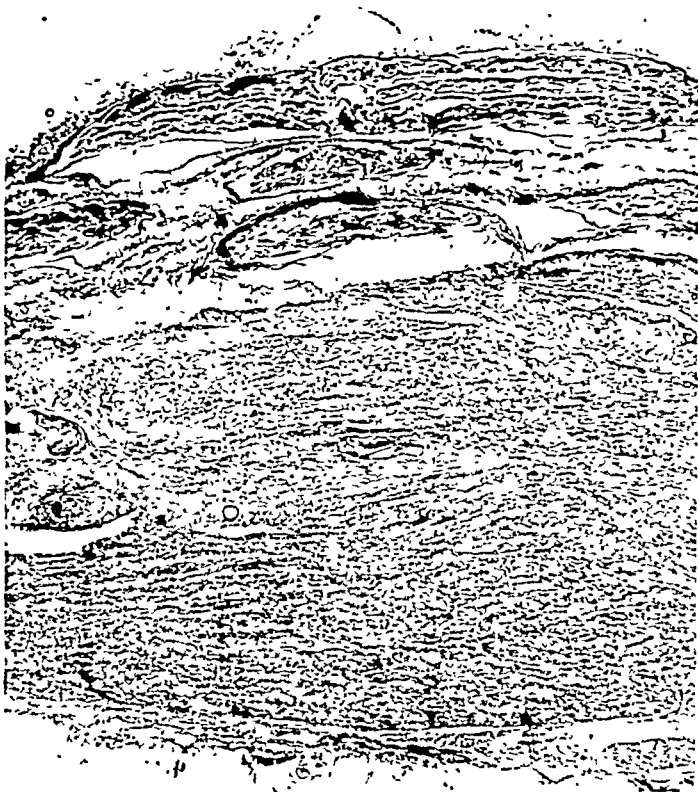


FIG. 2. HEMATOXYLIN AND EOSIN  $\times 30$

There was some weakness of supination. Marked atrophy of all the extensor muscles was present. No subjective or objective sensory changes were made out. No lesion could be seen or palpated over the course of the radial nerve or its deep branch. The diagnosis of a progressive lesion of the deep motor branch in the region of the supinator muscle was made by Dr. Frank Ford, who referred the patient to me.

Exploration of the nerve was carried out on April 28, 1945, exposure being made along the ulnar aspect of the brachioradialis muscle (Fig. 1). The main trunk of

the radial nerve was followed distally until the superficial (sensory) branch could be distinguished from the deep motor branch. Just below the division, on the motor branch, could be seen and felt a tiny nodule on the nerve, not more than 2 mm. in diameter. It was taken to be a neuroma. There was nothing in the

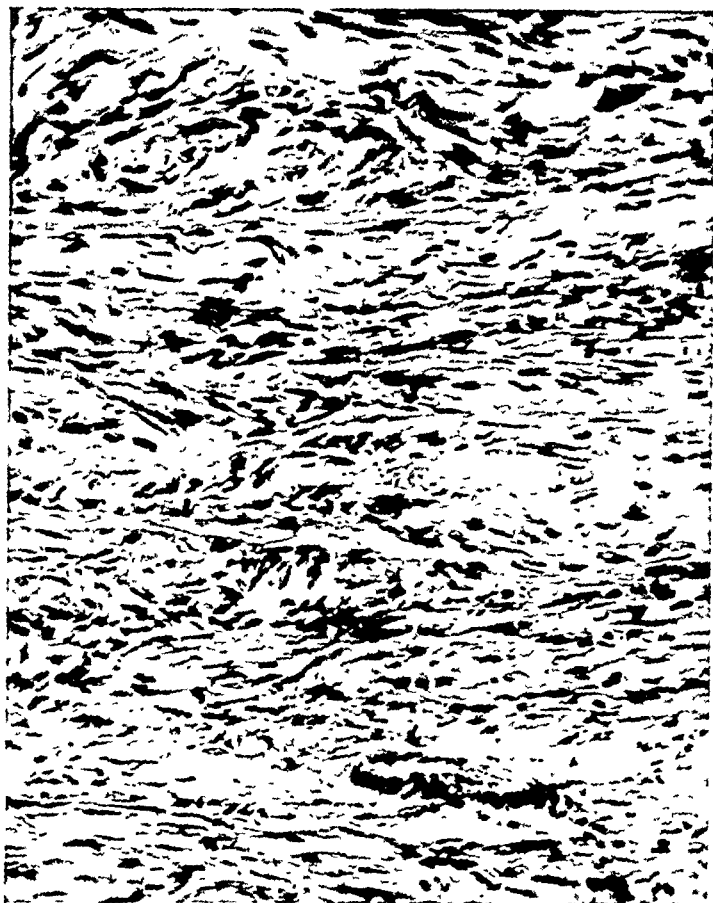


FIG 3. HEMATOXYLIN AND EOSIN  $\times 300$

external appearance of the nerve or its sheath to suggest the possibility of a previous trauma. The tumor was resected and the nerve sutured in continuity.

Microscopical study was made by Dr. Sam Blackman, whose notes are as follows: "Microscopically, along one margin of the specimen, there is a longitudinal section of a normal branch of the nerve (Fig. 2). The trichrome stains show the myelin sheaths and the axis cylinders. The larger branch of the nerve, however, is largely composed of connective tissue which is very cellular, but also contains many collagen fibers (Fig. 3). In the trichrome stain it is difficult to find

axis cylinders or myelin sheaths. This lesion differs from a neuroma in that it consists chiefly of a new growth of connective tissue. It might be considered either a scarred nerve or a fibrous tumor of the nerve. There is no history of trauma, and the absence of inflammation together with the history is some evidence against the lesion being a scar. It seems probable that it is a fibroma, although it is not at all a typical plexiform fibroma."

At the time of writing (two years after operation) there is no sign of return of function.

#### COMMENT

The history and physical findings in this case correspond very accurately to those in the five cases reported by Woltman and Learmonth (7), except that in the latter the weakness usually involved the fifth finger first, whereas in the present case, the thumb was affected first. In every case the extensor paralysis was progressive, there was marked atrophy and there were no subjective or objective sensory changes. The lesion had to be limited to the n. interosseus dorsalis. The unique anatomical course of the nerve through the supinator muscle has led to the assumption that some mechanical factor involved in this muscle-nerve relationship has resulted in the loss of function of the nerve. The present case illustrates the occurrence of an etiologic factor which is neoplastic and bears only an incidental relationship to the supinator muscle. It suggests that early diagnosis and surgical exploration of the nerve may lead to restoration of function.

#### BIBLIOGRAPHY

1. KOCH, S. L.: Injuries of the radial nerve. *Quart. Bull. Northwestern Univ. M. School*, 17, 1, 1943.
2. MOWELL, J. W.: Posterior interosseus nerve injury. *Internat. Clin.*, 2, 188, 1921.
3. MARIE, P., MEIGE, H., AND PATRIKIOS: Paralyse radiale dissociée simulant une griffe cubitale. *Rev. Neurol.*, 31, 132, 1917.
4. ROUSSY, G., AND BRANCHE, J.: Deux cas de paralysies dissociées de la branche postérieure du radial, à type de pseudo-griffe cubitale. *Rev. Neurol.*, 32, 312, 1917.
5. GRIGORESCO, D., AND IORDANESCO, G.: Un cas rare de paralysie partielle du nerf radial. *Rev. Neurol.*, 56, 102, 1931.
6. GUILLAIN, G., AND COURTELLEMENT: L'action du muscle court supinateur dans la paralysie du nerf radial. Pathogénie d'une paralysie radiale incomplète chez un chef d'orchestre. *Presse Méd.*, 1, 50, 1905.
7. WOLTMAN, H. W., AND LEARMONTH, J. R.: Progressive paralysis of the nervus interosseus dorsalis. *Brain*, 57, 25, 1934.

# THE MECHANISM OF EXCRETION OF AMMONIUM THIOSULFATE\*

JOHN FRANKLIN†, JACQUES GENEST AND ELLIOT NEWMAN

With Technical Assistance of MARGOT ROBINSON and MARION BIRMINGHAM

*From the Physiological Division, Department of Medicine, The Johns Hopkins University  
and Hospital, Baltimore, Maryland*

Thiosulfate has been extensively employed in the treatment of heavy metal and cyanide poisonings and is reported to have therapeutic value in certain vascular diseases. This background of frequent clinical use has revealed that the drug possesses no serious toxic actions. It is an old observation that thiosulfate is readily excreted by the kidney and its use as a measure of renal function was suggested by Nyiri in 1923 (1).

Gilman and associates (2) in the course of studies on the antidotal value of thiosulfate against chemical warfare agents, found in dogs that the renal clearance of thiosulfate was identical to the creatinine clearance or the glomerular filtration rate. In other words, the thiosulfate ion is excreted only by the glomerulus, a fact which labels it as the only inorganic ion which is known to be excreted independently of tubular function.

Newman, Gilman and Philips (3) extended the studies on thiosulfate clearance to the human kidney and concluded that thiosulfate and inulin clearances are identical. The criteria for establishing sodium thiosulfate as a measure of glomerular filtration rate have therefore been fulfilled.

Because of the unique mechanism of excretion of this electrolyte it could be expected that the excretion of thiosulfate at the rate of glomerular filtration would obligate an equal rate of excretion of base. Preliminary studies in patients of the partition of urinary base excreted with the thiosulfate anion revealed that the total thiosulfate excretion was covered mainly with sodium except in a few individuals with diseased kidneys.

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† Clinical Fellow, American College of Physicians 1946-47.

The present study was undertaken to determine the effectiveness of thiosulfate in removing extracellular sodium in the dog, as well as its effect on the pattern of excretion of other electrolytes. For this purpose the ammonium salt was used to present the kidney with the thiosulfate anion without added sodium in order to obligate the removal of intrinsic extracellular sodium.

#### METHODS

Four types of experiments were performed on adult female dogs weighing from 13 to 20 kilograms kept on a constant weighed diet with water ad lib.

1. A short experiment in which ammonium thiosulfate<sup>1</sup> was administered intravenously for one hour and the pattern of urinary electrolyte excretion studied during the infusion and post infusion periods.

2. An exhaustive experiment in which ammonium thiosulfate was administered continuously intravenously until exitus of the dog, the urine and serum being analyzed at frequent intervals for changes in the partition of electrolytes.

3. A single dose oral experiment in which the urine was studied for a follow up period of seven hours.

4. A chronic experiment in which the drug was administered orally to two dogs for a period of three and a half days and the urine studied for an interval sufficiently long to provide pre- and post-experimental controls.

The dogs were kept in metabolic cages, and daily records were maintained of the dog's weight, temperature, food and water intake and urinary output.

Urine collections in short experiments were made by catheter and the bladder washed with sterile water to insure complete collection. Twenty four hour collections were completed by catheterization every morning. Acetic acid and thymol were used as preservatives in the 24 hour urine collection bottles.

A commercially prepared diet was fed which upon chemical analysis was found to provide the following:

Total N .....	4.0 gms./100 gms. diet
Sodium .....	12.3 meq./100 gms. diet

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<sup>1</sup>Supplied by Winthrop Chemical Company; Mr. J. R. Lucas, Baltimore.



Potassium.....	11.7 meq./100 gms. diet
Phosphate.....	78.0 meq./100 gms. diet
Chlorides.....	11.7 meq./100 gms. diet

In addition the diet provided adequate amounts of carbohydrate, fat and other essential minerals. The animals gained weight during control periods and never revealed evidences of a dietary deficiency.

The chemical analyses of urine and serum involved minor modifications of accepted quantitative techniques.

Sodium and potassium determinations on both urine and serum were made by the flame photometer (4). This method was adequately tested by Hald and Peters (5) and compared with standard quantitative chemical techniques. Our equipment has been similarly checked and sufficient duplicate determinations have been made to assure us that readings are reproducible (6).

The Harvey (7) modification of the standard Volhard (8) titration method for chlorides was further modified because addition of nitric acid to urine containing thiosulfate caused precipitation of colloidal sulfur and organic matter, which made the end point difficult to read. The addition of enough nitric acid to precipitate the thiosulfate, followed by thorough clearing with permanganate, before the addition of the standard nitric acid-silver nitrate solution, eliminated the interfering color.

Similarly the standard Folin (9) method for the quantitative determination of total sulfate (etheral plus inorganic sulfate) was modified because of the presence of thiosulfate. The latter was precipitated from the urine as colloidal sulfur by the addition of nitric acid and heating. The sulfur was then removed by addition of infusorial earth and filtering. An aliquot of the filtrate was used for sulfate determination.

Phosphates were determined by the method of Fiske and Subbarrow (10), and thiosulfate in urine and serum by the method of Newman, Gilman and Philips (3). The Albanese (11) method and apparatus were employed in ammonium determinations. Total proteins were determined by the Macro Kjeldahl technique, and NPN by the Buell (12a) modification of the Kock, McMeekin (12) method. Gentzkow's (13) method for urea nitrogen was employed.  $\text{CO}_2$  combining power was determined by the volumetric method of Van Slyke (14).

The amount of ammonium thiosulfate employed is given in the dis-

cussion of the separate experiments. When given orally it was powdered and put in gelatin capsules.

### RESULTS

The effect of short intravenous administration of ammonium thiosulfate is illustrated in Figure 1. To a dog weighing 17.35 kilograms on 500 grams of diet, 2.7 grams of ammonium thiosulfate were administered in an isotonic infusion during the first hour and urine collections were made at hourly intervals for a total of four hours. The last column in Figure 1 represents the hourly rate of excretion of electrolytes during the remainder of the 24 hour period.

The total urine volume for this day was 1190 cc.; an increase of 400 cc. over the average daily output. The hourly urine volume follows closely the pattern of total sodium excretion.

There is a slight increase in ammonia excretion which may be due to stimulation by the acid ion of the base-saving ammonia-producing mechanism of the kidney.

The increase in sodium excretion is most striking. 40 Meq. of sodium appeared during the four hour experimental period whereas the total excretion for the day was only 47 Meq. The 7 Meq. excreted during the post experiment period indicates that a compensatory retention of sodium occurred following the large initial loss. The total excretion of sodium for the day was not greatly increased above the average because of the short duration of the experiment, but  $\frac{2}{3}$  of this daily total was excreted in four hours.

There is a slight but delayed increase in potassium excretion.

Thiosulfate occupies the largest portion of the anion block and represents a total excretion of 31 Meq. or a total recovery of 81% of the administered drug. The  $\text{SO}_4$  excretion is increased above the average daily excretion sufficiently to account for that portion of thiosulfate not recovered. If one adds the portion of  $\text{SO}_4$  which represents oxidized thiosulfate to the thiosulfate block, the block representing sodium excretion is practically superimposable or equal.

Chloride and phosphate ions were excreted in negligible amounts during the experimental period, appearing after the kidney had cleared the thiosulfate. The total 24 hour excretion of chloride on the day of experiment was decreased.

The pattern of electrolyte response to ammonium thiosulfate in both

urine and serum when the drug was administered intravenously until exitus of the animal is shown in Figure 2.

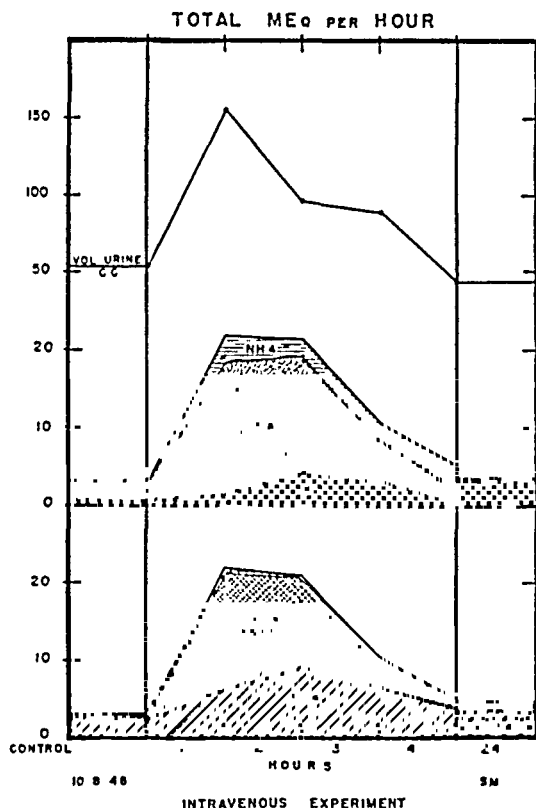


FIG. 1. THE EFFECT OF INTRAVENOUS ADMINISTRATION

Dog SM. Fasting, Unanesthetized, Wt. 17.35 kilograms. Diet 500 grams daily. 2.7 grams ammonium thiosulfate intravenously as isotonic infusion during first hour. Urine collections hourly for four hours. Control hour and hourly rate of excretion of remaining 24 hours shown. Ordinate is total milliequivalents.

A total of approximately 25 gm. of ammonium thiosulfate was given by constant infusion to a dog weighing 17 kilograms until death occurred in six hours.

The changes in serum are represented as increments above or below a base line of control values.

The pattern of electrolyte response in the urine is similar to the short intravenous experiment, with a striking increase in sodium excretion



On the anion side, thiosulfate occupies the major portion of the block and represents a total of 13 gm. of recovered drug.  $\text{SO}_4$  is increased as in the short experiment. Chloride excretion was minimal and no phosphate was recovered in the urine.

The decreased urine volume during the last collection period can be correlated with other evidence of renal failure; first, a decreased glomerular filtration rate as measured by thiosulfate clearance and second, a rising NPN and urea N. The concentrations of urea and non protein nitrogen rose, particularly terminally, to levels of 57 and 64 milligrams per cent, respectively.

Most important in this experiment are the serum changes which indicate the probable mechanism of death. The serum sodium concentration fell from 144 to 125 and the serum potassium rose from 4.4 to 12 milliequivalents per liter. With these striking changes there was a rise of serum chloride of 25 Meq. and a fall of the  $\text{CO}_2$  combining power from 23.3 to a final level of 4 Meq. per liter.

Evidence of hemoconcentration and loss of extracellular fluid is shown by the rise in the hematocrit and the total serum protein concentration. One might at first conclude that the loss of extracellular fluid causing the signs of hemoconcentration was due to a marked loss of water with the thiosulfate diuresis. Actually there was no overall loss of body water in this experiment since the water administered in the infusion of thiosulfate approximately equalled the urine output.

If the contraction of extracellular volume was not due to negative fluid balance, an alternative explanation is that the marked loss of sodium with lowered sodium concentration caused a shift of water into muscle cells. Analysis of the water content of this dog's muscle by Dr. Kenneth Zierler showed an increase to 89% water, normal being 76%.<sup>2</sup> Concomitant with the shift of water from extracellular fluid into cells it is likely that cell potassium escaped, thus accounting for the marked increase in serum potassium to a level which is usually lethal. The phenomenon of shift of extracellular water into cells with a release of potassium in the other direction due to marked loss of sodium has been the subject of much recent experimental and clinical study by Darrow, Finch and others (15, 16).

<sup>2</sup> Determined by difference between whole wet weight and dry weight.

At autopsy, no gross or microscopic pathology was seen in the heart, kidneys or adrenal glands of this dog.<sup>3</sup>

In Figure 3 is illustrated the pattern of electrolyte excretion in response to 5 gm. of ammonium thiosulfate administered orally to a dog

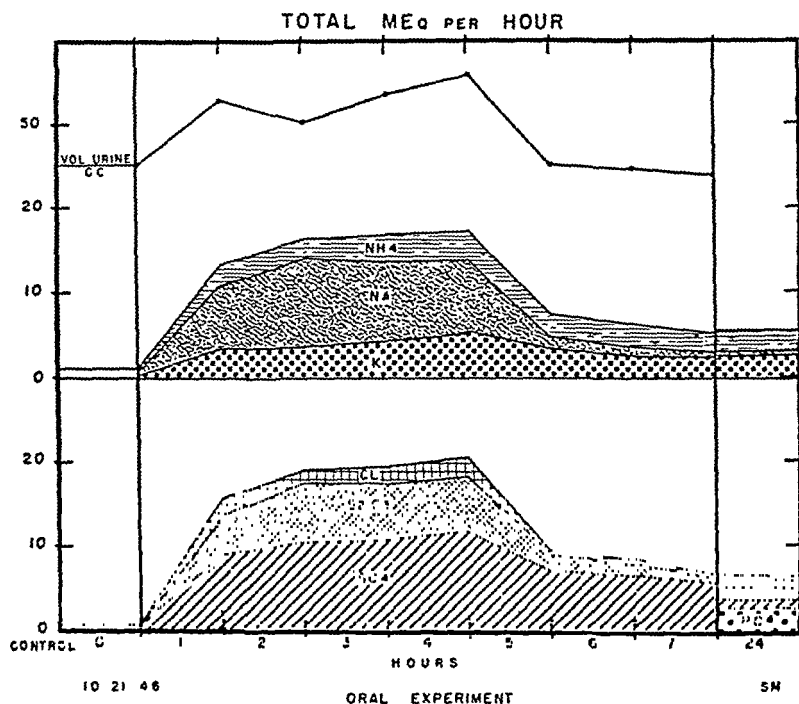


FIG. 3. THE EFFECT OF A SINGLE ORAL DOSE

Dog SM, Fasting, Unanesthetized, Wt. 17.75 kilograms, diet 500 grams daily. 5 grams ammonium thiosulfate in 20 cc. of water by duodenal tube. Control hour total electrolyte excretion shown and hourly total electrolyte excretion in milliequivalents for seven hours following administration. Hourly rate of excretion for remaining 17 hours shown. Hourly urine volume in cc.

weighing 17.75 kilograms on 500 grams of diet daily. The urine was collected at hourly intervals for seven hours.

The pattern of response is similar to that illustrated in Fig. 1 with an increase in ammonia, and a dramatic increase in sodium excretion,

<sup>3</sup> Sections examined by Dr. Mitchell Miller, Instructor in Pathology.

followed by a post experiment period of sodium retention. There is a delayed increase in potassium excretion.

The rate of thiosulfate excretion is slower than in the intravenous experiment. The total recovered was 32 Meq. or 48% of the amount administered. The greatly increased  $\text{SO}_4$ , a total of 84 Meq., is sufficient to account for that portion of thiosulfate not recovered as such, and indicates that after oral administration approximately half of the administered thiosulfate is oxidized to  $\text{SO}_4$ .

No phosphate was excreted during the experiment period and chloride excretion was not as low as after intravenous administration of the drug.

Again the sodium block, and thiosulfate plus sulfate blocks are superimposable. The increase in urine volume follows closely the pattern of increased sodium excretion.

In Figure 4 is summarized the control data on one dog for twenty five days during which time two intravenous experiments and one oral experiment were done. On day 1 five grams and on day 8 two and a half grams of ammonium thiosulfate were given intravenously. On day 21, five grams were given orally. The general pattern of response on experiment days consists of increased urine flow; low chloride excretion; increased sulfate excretion particularly after oral administration; slight increase in ammonia excretion; little significant potassium increase; and finally an increase in sodium excretion followed by sodium retention particularly after the larger intravenous dose on day 1.

On the whole the total electrolytes excreted in 24 hour periods do not show marked increases except for sulfate because the duration of the administration was only for a fraction of the day, so that compensatory retention for the remainder of the day largely balanced the early loss.

Other variations in urine volume and electrolyte excretion are the usually spontaneous variations of a dog on a constant diet with water ad lib.

The effects of chronic oral administration of ammonium thiosulfate were studied in two dogs and results are illustrated in Figures 5 and 6. Each received a total of 35 grams of the drug over a period of three and a half days.

The total recoveries of thiosulfate in urine in these experiments were 85 and 93 per cent in Figures 5 and 6 respectively. Of the total re-

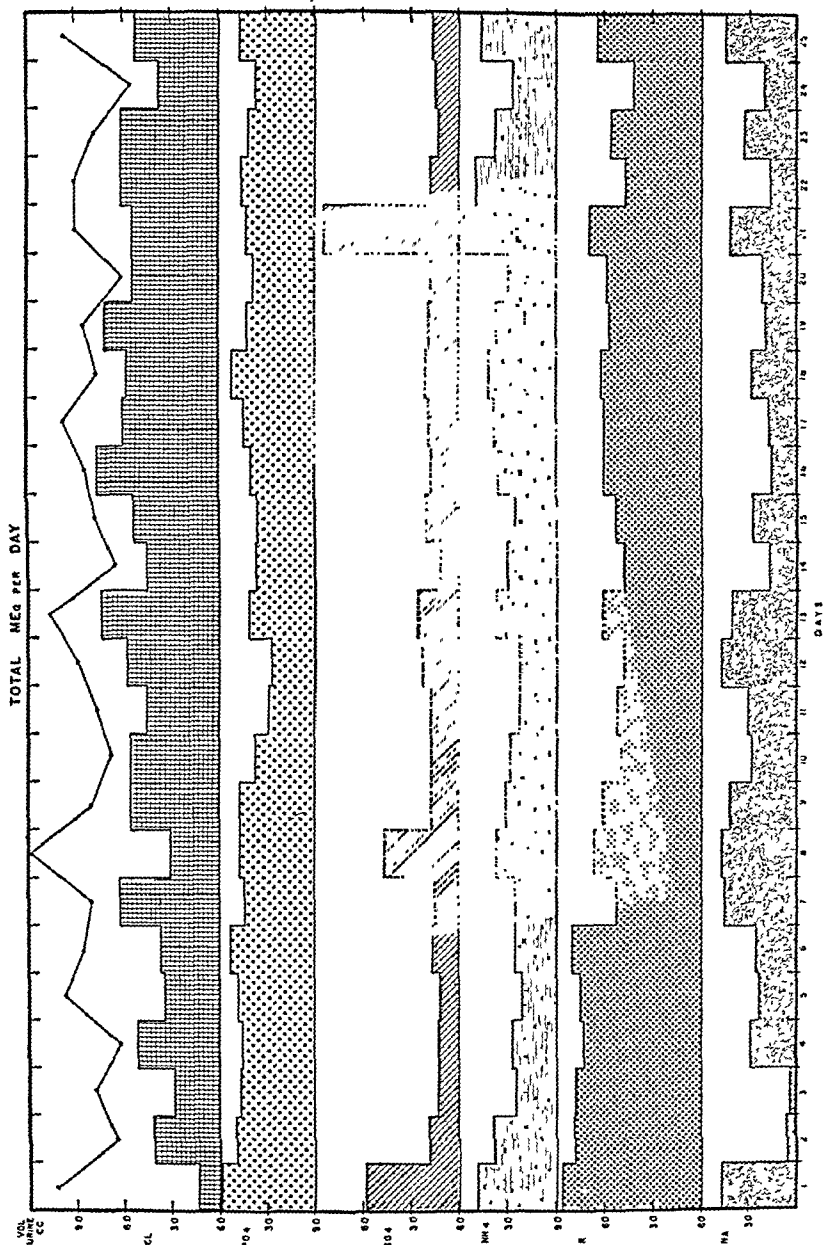


FIG. 4. Illustrating routine control 24 hour determinations of total electrolytes in milliequivalents and the effect on sulfate excretion of intravenous ammonium thiosulfate on days 1 and 8 and oral ammonium thiosulfate on day 21. Dog SM, Wt. 18 kilograms, Diet 500 grams daily.



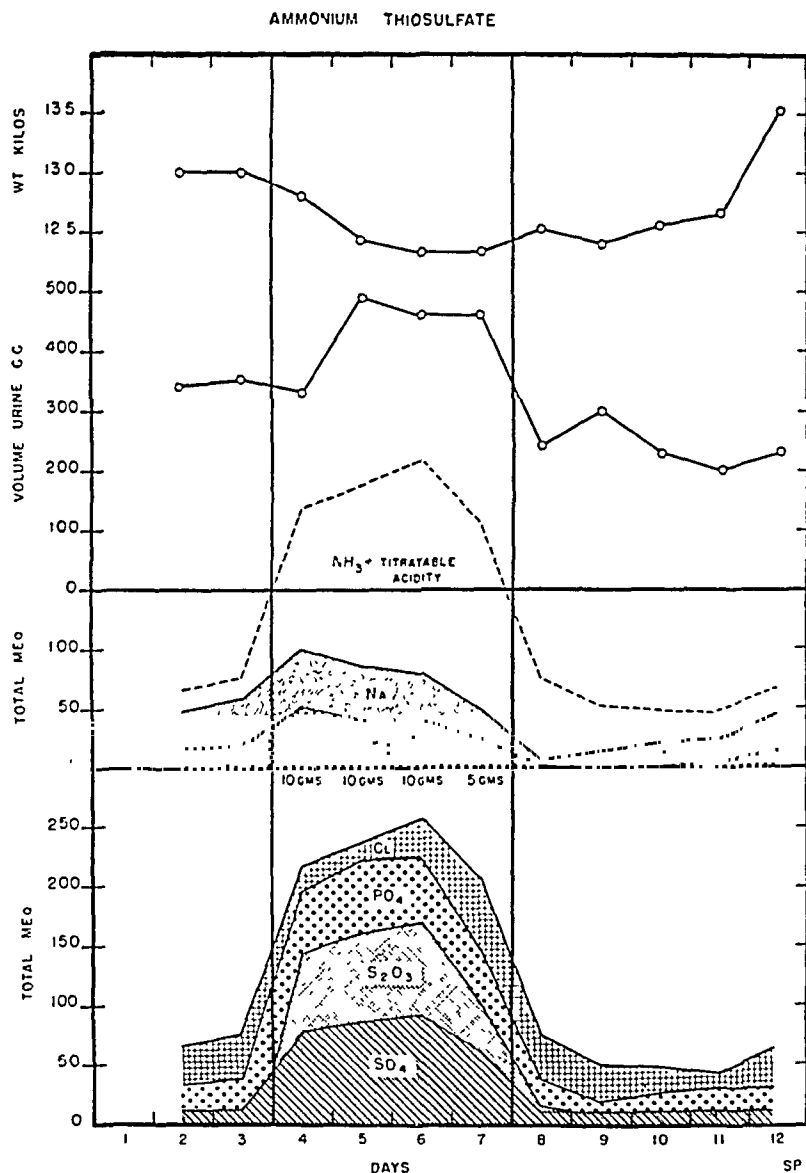


FIG. 5. Oral administration of 35 grams of ammonium thiosulfate in four days. Two days control and four days following administration recorded. Dog SP, Wt. 13.0 kilograms, Diet 300 grams daily.

## AMMONIUM THIOSULFATE

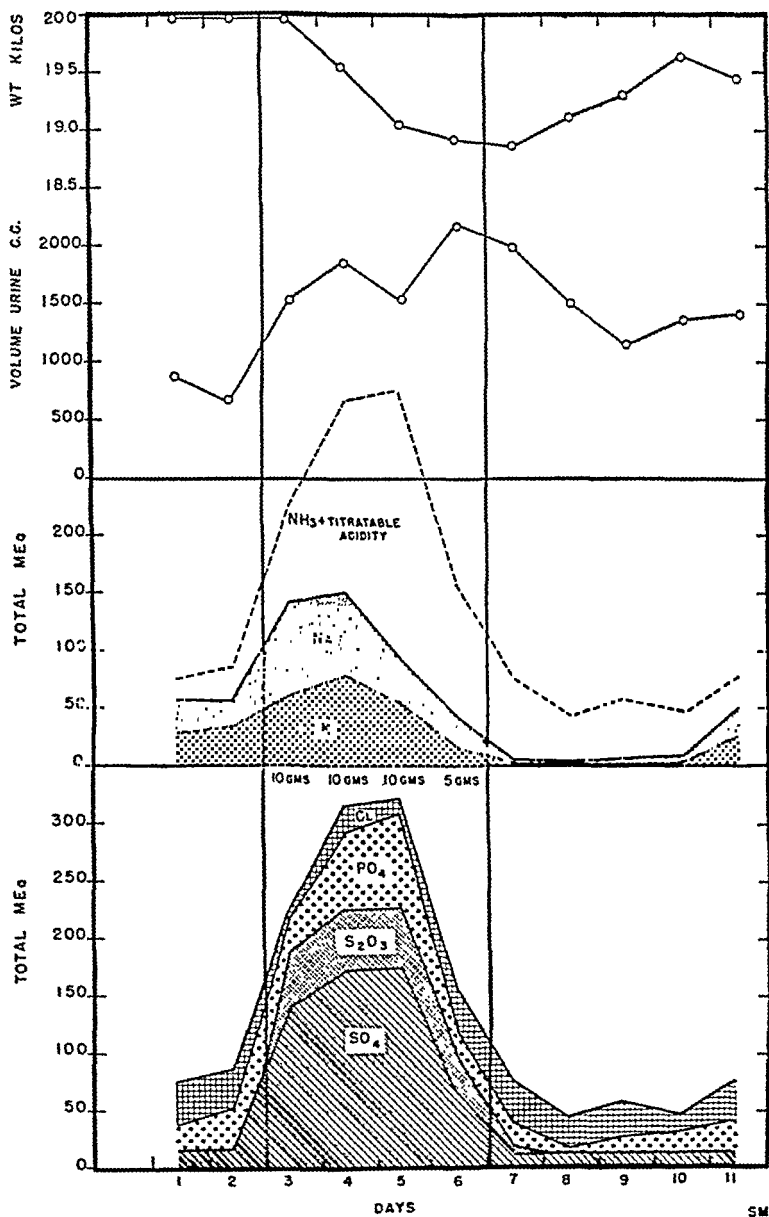


FIG. 6. Oral administration of 35 grams of ammonium thiosulfate in four days. Two days control and four days following administration recorded. Dog SM. Wt. 20 kilograms, Diet 250 grams daily.

covered 44 to 66 per cent was present as the thiosulfate ion in the urine and the balance as the sulfate ion. As thiosulfate yields two sulfate ions upon oxidation, the larger sulfate excretion is thus explained.

The pattern of sodium and potassium excretion is similar to but less dramatic than in the previously described experiments. The dog in Figure 6 doubled her average daily sodium excretion during the period of drug administration, excreting a total of 107 Meq. of sodium in excess of an amount calculated from the preceding control period. The total potassium lost during the experiment almost equaled the sodium loss, being 92 Meq. in excess of the control excretion.

The dog in Figure 5 excreted less sodium with the same amount of the drug, her total excess being only 30 Meq., but potassium excretion was significantly increased over the control excretion to 80 Meq.

The difference in sodium loss in the two dogs is not accounted for on the basis of dose-body weight relationship since dog No. 1 which lost more sodium was heavier and received an equal amount of the drug. The amount of the post-administration retention of sodium was roughly proportional to the amount lost during administration of the drug. Another factor affecting the difference in sodium loss in the two dogs was probably the difference in the amount of ammonia formation.

The total anion excretion was quite large and was undoubtedly largely balanced by ammonia. Although ammonia was not directly determined, the calculated total represents a major portion of the cation block, particularly in Figure 5. Chronic oral administration of ammonium thiosulfate apparently stimulates the ammonia-producing mechanism of the kidney. Thus part of the value of this drug as a remover of fixed base is vitiated. However, the fact that both of these dogs lost weight during the period of drug administration is evidence that the drug was effective in removing intrinsic body base and water.

The marked base saving due to ammonia production in these chronic experiments is in contrast to the marked sodium loss during the short intravenous administrations. Apparently significant stimulation of the ammonia-producing mechanism does not take place within a few hours, but increases rapidly in the course of three days.

There was increased renal tubular reabsorption of chloride during the first two to three days of drug administration at a time when sulfate excretion rose to levels representing a six to ten fold increase over the

base line control excretion. This illustrates the phenomenon of increased reabsorption of chloride in the presence of a large increment of sulfate. Chloride excretion subsequently rose as sulfate excretion decreased at the end of the experiment period.

Contrasting with the very low phosphate excretion observed in the intravenous experiments, phosphate excretion rose sharply in these dogs. This is particularly evident in Figure 5 where the 24 hour total for phosphates is double that of the control period. A post-experiment suppression of phosphate excretion also occurred.

The increased phosphaturia can be partially correlated with the rise in potassium excretion although the latter is of greater magnitude. Phosphaturia in response to the rapid development of acidosis particularly following the administration of acid or acid producing substances is an old observation (17). Rappoport and coworkers have extended this observation by identifying diphosphoglycerate, an organic acid soluble phosphorus in blood cells, as the probable source of part of the increased inorganic phosphaturia (18). It is possible that the decomposition of diphosphoglycerate in the blood cell in response to acidosis is similar to processes occurring in other tissues.

#### DISCUSSION

The results obtained after administration of ammonium thiosulfate are similar in many respects to the effects of other ammonium salts of inorganic acids. The loss of sodium and potassium with increase in urine output and loss of body weight are the effects desired therapeutically when these salts are used as diuretics in patients with edema.

The early studies of Blum, Haldane, Keith and Gamble (19, 20, 21, 22, 23) revealed the mechanism of diuretic action of ammonium chloride, calcium chloride and ammonium sulfate. Ammonium nitrate has been used by Keith and others (24, 25, 26) but abandoned because of cyanosis and methemoglobinemia due to the reduction of some nitrate to nitrite.

The effectiveness of ammonium salts as diuretics or "base-removers" has been shown to be limited mainly by the capacity of the kidney to secrete ammonia, thereby saving base. More loss of base occurs at the beginning of administration because ammonia production is slow in starting and does not reach a maximum for three days in the normal

kidney. In damaged kidneys, the loss of base is greater due to failure to produce ammonia (27, 28). Ammonium thiosulfate stimulates ammonia production in a similar manner in the dog.

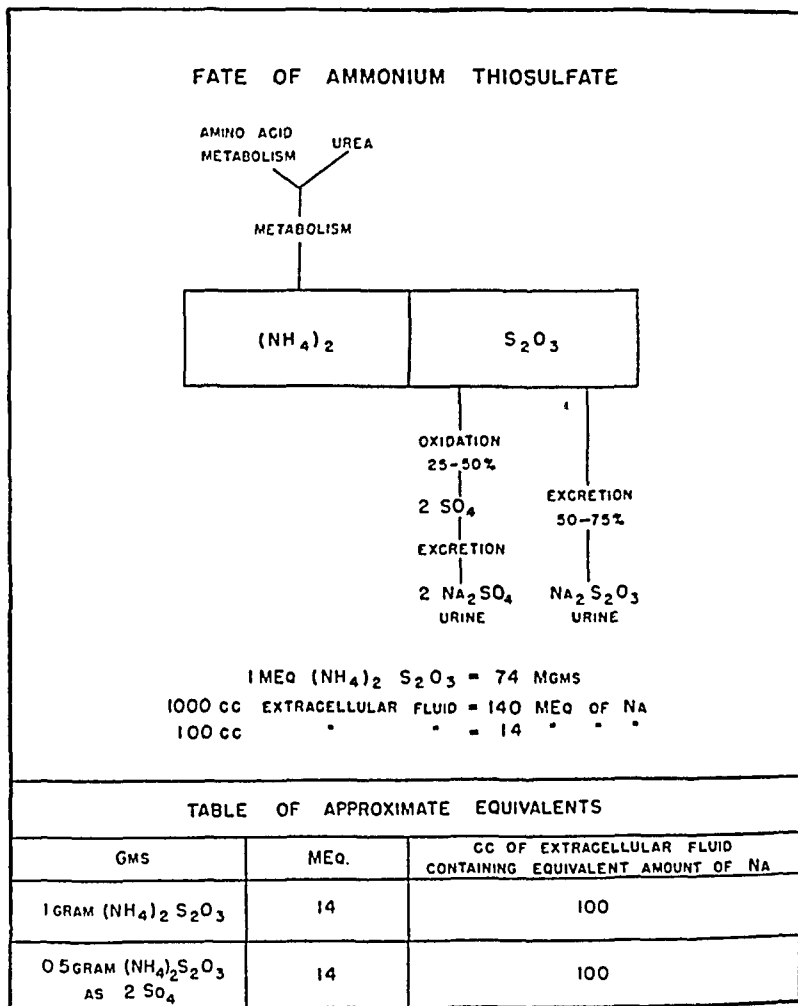


FIG. 7

Toxic effects have been attributed to the "acid producing" salts. Besides acidosis and dehydration produced by prolonged administration, minor toxic symptoms have been noted. Calcium chloride and

the ammonium salts of chloride, sulfate and nitrate produce some nausea and vomiting. Occasional vomiting occurred in our dogs after several days on large doses of ammonium thiosulfate.

The thiosulfate ion has two properties which are unique and different physiologically from the anions of other diuretic ammonium salts. First, thiosulfate is the only known inorganic electrolyte which is not reabsorbed by the renal tubules so that in both dogs and humans it is as rapidly excreted as any electrolyte can be, assuming that no inorganic electrolytes are secreted by the renal tubule. Thiosulfate, therefore, has the advantage as a "base-remover" that the rapidity of excretion is governed only by the rate of glomerular filtration after intravenous administration or after absorption from the gut. Like other acid salts its effectiveness is limited by the capacity of the kidney to secrete ammonia.

Another property of thiosulfate as an "acid salt diuretic" is derived from the process of conversion of some thiosulfate to sulfate, as illustrated in Figure 7. In dogs about 25% of intravenously and 50% of orally administered thiosulfate is converted to sulfate. Since one thiosulfate ion upon oxidation yields two sulfate ions, the potential base removing power of thiosulfate is doubled by the conversion. Furthermore the excess sulfate is excreted almost as rapidly as thiosulfate. The rapid excretion of sulfate can be seen in the charts and is confirmed by the fact that during thiosulfate excretion enough sulfate was recovered to account for the portion of thiosulfate which was oxidized. Rapid excretion of excess sulfate by the kidney has been described by Winkler after injections of sodium sulfate (29).

#### SUMMARY

Adult female dogs were given ammonium thiosulfate orally and intravenously to determine the effect on electrolyte excretion of an anion which is not reabsorbed by the renal tubules.

After a single intravenous injection of ammonium thiosulfate, approximately three fourths of the thiosulfate was recovered in the urine within three hours and the remaining one fourth was recovered as sulfate.

After a single oral dose of ammonium thiosulfate half of the thiosulfate was recovered in the urine and half as sulfate within seven hours.

The rapid excretion of thiosulfate after single doses is accompanied by a large increased sodium and slightly increased potassium excretion. With repeated doses less sodium is lost and larger amounts of ammonia are excreted. After single doses the excretion of chloride and phosphate is very low; but with repeated doses there is low excretion of chloride and a marked increase in phosphate excretion.

Increases in urine flow occurred regularly and followed the pattern of increased total base excretion. With repeated doses there was weight loss.

Continuous administration intravenously for six hours caused death with a low serum sodium and a high serum potassium.

Some vomiting occurred with large repeated oral doses in dogs.

#### BIBLIOGRAPHY

- (1) NYIRI, W.: *Über die Thiosulfatprobe eine neue methode der nierenfunktionsprüfung.* Klin. Wchnschr., 2(1): 204, 1923.
- (2) GILMAN, A., PHILIPS, F. S., AND KOELLE, G. S.: *The Renal Clearance of Thiosulfate with Observations on its Volume Distribution.* Am. J. Physiol., 146: 348, 1946.
- (3) NEWMAN, E. V., GILMAN, A., PHILIPS, F. S.: *The Renal Clearance of Thiosulfate in Man.* Bull. Johns Hopkins Hosp., 79: 229, 1946.
- (4) BARNES, R. B., RICHARDSON, D., BERRY, J. D., AND HOOD, R. L.: *Flame Photometry, a Rapid Analytical Procedure.* Industrial and Engineering Chemistry, Analytical Edition, 17: 605, 1945.
- (5) HALD, PAULINE M.: *The Flame Photometer for the Measurement of Sodium and Potassium in Biological Materials.* J. Biol. Chem., 167: 499, 1947.
- (6) HOWARD, J. E., AND BIGHAM, R. S., JR.: *Minutes of the Conference on Metabolic Aspects of Convalescence,* New York. Josiah Macy, Jr. Foundation, Eleventh Meeting, Oct. 15-16, 1945.
- (7) HARVEY, S. C.: *The Quantitative Determination of Chlorides in the Urine.* Arch. Int. Med., 6: 12, 1910.
- (8) VOLHARD, J.: *Die Silberfärbung mit Schwefelcyanammonium.* Z. Anal. Chem., 17: 482, 1878.
- (9) FOLIN, O.: *On Sulphate and Sulphur Determinations.* J. Biol. Chem., 1: 131, 1905.
- (10) FISKE, C. H., AND SUBBARROW, Y.: *The Colorimetric Determination of Phosphorus.* J. Biol. Chem., 66: 375, 1925.
- (11) ALBANESE, A. A.: *Determination of Ammonium in the Urine.* J. Lab. & Clin. Med., 29: 447, 1944.
- (12) KOCK, F. C., AND MCMEEKIN, T. L.: *A New Direct Nesslerization Micro-Kjeldahl Method and a Modification of the Nessler-Folin Reagent for Ammonia.* J. Amer. Chem. Soc., 46: 2066, 1924.

- (12a) BUELL, M. U.: Personal Communication.
- (13) GENTZKOW, C. J.: An Accurate Method for the Determination of Blood Urea  $N_2$  by Direct Nesslerization. *J. Biol. Chem.*, **143**: 531, 1942.
- (14) VAN SLYKE, D. D., AND STADIE, W. C.: The Determination of Gases of the Blood. *J. Biol. Chem.*, **49**: 1, 1921.
- (15) DARROW, D. C.: The Retention of Electrolyte during Recovery from Severe DEHYDRATION due to Diarrhea. *J. Pediat.*, **28**: 515, 1946.
- (16) FINCH, C. A., SAWYER, C. G., FLYNN, J. M.: Clinical Syndrome of Potassium Intoxication. *Am. J. Med.*, **1**: 337, 1946.
- (17) FORBES, E. B., AND KEITH, M. H.: A Review of the Literature of Phosphorus Compounds in Animal Metabolism. Wooster, Ohio Agricultural Experiment Station, Technical Series Bull., **5**: 510, 1914.
- (18) GUEST, G. M., AND RAPPOPORT, S.: Organic Acid-Soluble Phosphorus Compounds of the Blood, *Physiol. Rev.*, **21**: 410, 1941.
- (19) BLUM, L., AUBELL, E., AND HAUSKNECHT, R.: L'Action Diuretique des sels de Calcium dans les Oedemes Generalises. *Bull. et mem. Soc. Med. d. Hop. de Paris*, **45**: 1561, 1921.
- (20) HALDANE, J. B. S.: Experiments on the Regulation of the Blood's Alkalinity. *J. Physiol.*, **55**: 265, 1921.
- (21) HALDANE, J. B. S., HILL, R., AND LUCH, J. M.: Calcium Chloride Acidosis. *J. Physiol.*, **57**: 301, 1923.
- (22) KEITH, N. M., AND WHELAN, M.: A Study of the Action of Ammonium Chloride and Organic Mercury Compounds. *J. Clin. Invest.*, **3**: 149, 1926.
- (23) GAMBLE, J. L., BALCKFAN, K. D., AND HAMILTON, B.: A Study of the Diuretic Action of Acid Producing Salts. *J. Clin. Invest.*, **1**: 359, 1925.
- (24) KEITH, N. M., WHELAN, M., AND BANNICK, E. G.: Diuretic Action of Nitrates and their Use in the Treatment of Dropsy. *Tr. Assoc. Am. Phys.*, **43**: 288, 1928.
- (25) KEITH, N. M., AND EUSTERMAN, G. B.: Transient Methemoglobinemia following Administration of Ammonium Nitrate. *M. Clin. North. Amer.*, **12**: 1489, 1929.
- (26) TARR, L.: Transient Methemoglobinemia due to Ammonium Nitrate. *Arch. Int. Med.*, **51**: 38, 1933.
- (27) PALMER, W. W., AND HENDERSON, L. J.: A Study of the Several Factors of Acid Excretion in Nephritis. *Arch. Int. Med.*, **16**: 109, 1915.
- (28) VAN SLYKE, D. D., LINDER, G. C., HILLER, A., LEITER, L., AND MCINTOSH, J. F.: The Excretion of Ammonia and Titratable Acid in Nephritis. *J. Clin. Invest.*, **2**: 255, 1926.
- (29) WINKLER, A. W., PETERS, J. P., AND ASSOCIATES: Duncan's Diseases of Metabolism. W. B. Saunders Co., Philadelphia and London, 1942, page 292.



# THE USE OF TELEVISION IN SURGICAL OPERATIONS

I. R. TRIMBLE AND F. M. REESE

*From the Departments of Surgery and Ophthalmology of the Johns Hopkins University, School of Medicine, Baltimore, Maryland*

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Television of surgical operations was used for the first time at the Johns Hopkins Hospital on 27 February 1947. The experiment was dramatically successful.

The customary method of demonstrating surgical operating technique to students and to visiting doctors or nurses has proved unsatisfactory for two reasons. In the first place, the observers cannot see the operating field properly; in the second place, these observers bring contamination into the operating room. Except for two or three people who stand on raised platforms immediately behind the operator, the demonstration of an operation is almost never satisfactory. To a class in surgery an operative clinic is almost a complete waste of time. Also it is a well established fact that the number of pathogenic organisms which may be cultured from the air of an operating room is in direct proportion to the number of people in the room even though these people are gowned, capped, and masked, and that the bacteria gain their entrance into the room mainly in the nose and throat passages of the people present. In order to overcome these difficulties in clinical instruction in surgery, the authors conceived the idea of employing television. When the Radio Corporation of America was consulted on the subject they immediately dispatched a representative to Baltimore from Camden, New Jersey, to consult about the proposition.

A stimulus to those studying the possibilities was the projected biennial meeting of the Johns Hopkins Medical and Surgical Association at which 750 members were scheduled to attend. Since a large percentage of these are surgeons, and since many others are interested in the newer surgical techniques, especially the so-called "blue baby" operations on the heart, it was foreseen that the operating rooms would be swamped with visitors. Accordingly, a target date was set with the Radio Corporation of America to make every possible effort to secure and set in place the necessary television equipment for use on the days of the meeting, 28 February-1 March, 1947.

Besides the procurement of this equipment there were certain engineering problems to be settled. These were the construction of a de-

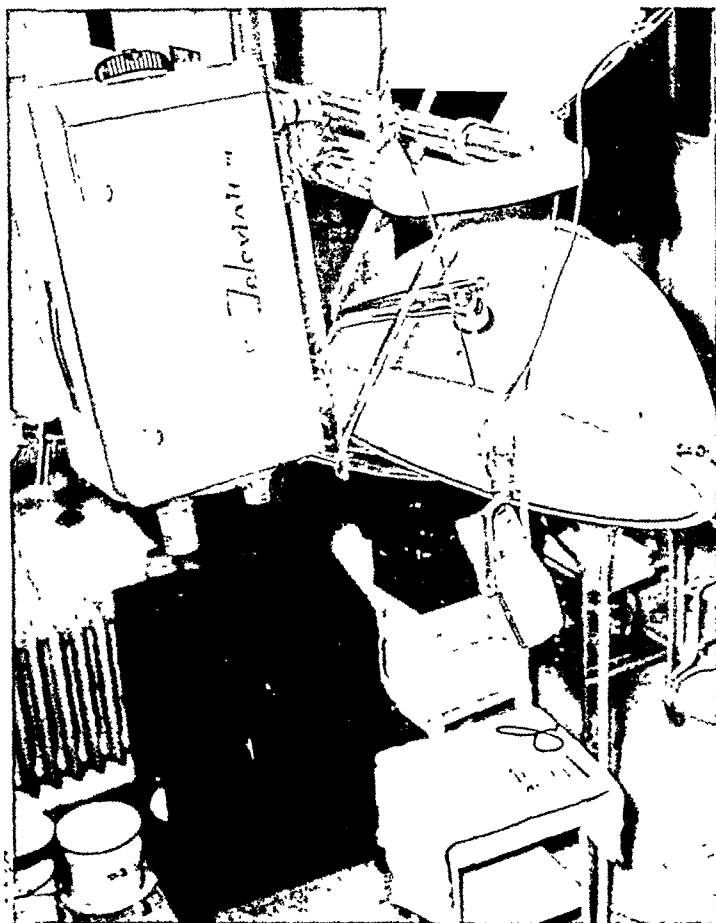


FIG. 1. TELEVISION CAMERA MOUNTED ON OPERATING ROOM CENTRAL LIGHT FIXTURE

Distance of camera above operating room table  $4\frac{1}{2}$  feet. Microphone apparatus in place

vice to hang the camera from the central overhanging electric light fixture in the operating room and the installation of two temporary single phase, alternating current, 30 ampere, 110 volt circuits, one for

the camera and the electronic transmission, and the other for the projectors.



FIG. 2. CHEST OPERATION FOR PULMONIC STENOSIS

Five operations were transmitted to ten television receivers in four different classrooms on two separate floors at distances of approximately 200 feet from the operating room. At the same time through a loud speaker system the surgeon described each operation step by step as he performed it.

Four of the operations were performed on the heart, the fifth on the sympathetic nerve trunk along the vertebral column. The first two operations were performed by Dr. Alfred Blalock using the technique



FIG. 3. ABDOMINAL OPERATION TO SEVER LUMBAR SYMPATHETIC TRUNK

devised by him and Dr. Taussig to increase the insufficient amount of blood reaching the lungs because of congenital stricture of the pulmonary artery, the so-called "blue baby" operation. The third operation was performed by Dr. I. R. Trimble when the lumbar sympathetic nerve trunk was resected along the vertebral column in order to improve the circulation in the leg. Dr. H. W. Scott, Jr., performed the



FIG. 4



FIG. 5

FIGS. 4 AND 5. SPECTATORS VIEWING TELEVISION SCREENS

fourth operation, ligating a congenital abnormal opening in the great vessels coming off the heart, the patent ductus arteriosus. Dr. W. P. Longmire, Jr., performed the fifth operation, which was again the Blalock-Taussig operation on the heart.

Two television cameras were in constant use. Before operations were commenced the camera recording the operating field, which was clamped to the overhead operating light  $4\frac{1}{2}$  feet above the operating table, was accurately focused. So sharp was the image that when a dollar bill was placed on the operating table for the purpose of focusing the estimated distance of the operating field from the camera, the serial numbers on the bill could be clearly read on the projection screens. A 135 mm. focal length lens gave a field of view of  $8\frac{1}{2} \times 11\frac{1}{2}$  inches at this distance. Each step in the operations was clearly recorded by television—the incision, the clamping of small bleeding vessels, the delicate suturing with fine silk of the blood vessels as the subclavian and pulmonary arteries were united, the demonstration of fine nerve fibers. The pictures were registered in black and white since technicolor is still in its experimental stage in television.

The second television camera was installed in the gallery of the operating room to show a general view of the activity in the operating room. From the small control room pictures which were taken by this second camera were at times flashed on the projection screens, alternating with camera number one which was recording a close-up view of the operative field only.

It is believed that this experiment in television has proved conclusively that demonstration of and instruction in surgical operations to a group of more than three or four people can be best accomplished by the use of television and that future operating room construction will be greatly modified because of the introduction of this method of teaching.

The authors are indebted to Mr. W. L. Lawrence and Mr. N. S. Bean and the crew of advisers and technicians from the Radio Corporation of America, to Miss Elizabeth W. Sherwood, Head Nurse of the General Operating Rooms at the Johns Hopkins Hospital, and to her staff of nurses, and to Mr. W. D. Witter, Chief Engineer of the Johns Hopkins Hospital, and his staff.

# PHYSIOLOGICAL STUDIES IN CONGENITAL HEART DISEASE<sup>1</sup>

## IV. MEASUREMENTS OF THE CIRCULATION IN FIVE SELECTED CASES

L. D. VANDAM, R. J. BING, AND F. D. GRAY, JR.

*From the Department of Surgery, Johns Hopkins University and the  
Johns Hopkins Hospital*

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In the preceding two papers of this series circulatory measurements of the tetralogy of Fallot and of Eisenmenger's complex have been presented (1, 2). It is the aim of the present communication to describe the diagnostic value of these physiological studies when applied to a variety of clinical problems.

The various procedures employed in these studies of congenital heart disease have been reported in detail in the first paper of this series (3). Most of the data were obtained by applying the Fick principle. Blood gas values substituted in the Fick equation were obtained by catheterization of the heart chambers and great vessels, by arterial puncture, and by analysis of respiratory gases. In presenting the results of case studies in these reports a number of terms describing blood volume flows have been employed. For purposes of clarity they will be redefined at this time.

Pulmonary capillary flow, the amount of blood flowing through the lung capillaries per unit time, is derived from the formula:

$$1. \text{ Pulmonary capillary flow} = \frac{\text{CO}_2 \text{ output (ml. per min.)}}{\text{CO}_2 \text{ content of blood entering pulmonary capillaries (vol. per cent)} - \text{CO}_2 \text{ content of blood leaving pulmonary capillaries (vol. per cent)}} \times 100$$

Pulmonary artery blood flow, the volume of blood flowing through the pulmonary valve into the pulmonary artery, is calculated from the formula:

$$2. \text{ Pulmonary artery flow} = \frac{\text{O}_2 \text{ intake (ml. per min.)}}{\text{O}_2 \text{ content of pulmonary vein blood (vol. per cent)} - \text{O}_2 \text{ content of pulmonary artery blood (vol. per cent)}} \times 100$$

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<sup>1</sup> This work was supported by a grant from the Commonwealth Fund.

When the oxygen content of pulmonary vein blood cannot be directly determined, a figure based on the assumption that pulmonary vein blood is fully oxygenated is substituted in this equation. Moreover, when a blood sample is not procurable from the pulmonary artery, the oxygen content of blood taken from the outflow tract of the right ventricle is likewise substituted for pulmonary artery blood in the equation.

The collateral pulmonary blood flow is that portion of the capillary flow to the lungs contributed by sources other than the pulmonary artery. Hence:

$$\begin{aligned} 3. \text{ Collateral pulmonary blood flow (ml. per min.)} = \\ \text{Pulmonary capillary flow (ml. per min.)} - \text{pulmonary artery flow (ml. per min.)} \end{aligned}$$

The systemic blood flow, the volume of blood coursing through the peripheral blood vessels, is expressed by the formula:

$$4. \text{ Systemic flow (ml. per min.)} = \frac{\text{O}_2 \text{ intake (ml. per min.)}}{\text{O}_2 \text{ content of peripheral arterial blood (vol. per cent)} - \text{O}_2 \text{ content of right auricular blood (vol. per cent)}} \times 100$$

The intracardiac shunt is the volume of blood per unit time shunted from one side of the heart to the other through septal defects. The shunt is designated "right to left" or "left to right" depending on the predominant direction in which the volume of blood is shifted. The formulae for calculation of the shunt are:

$$\begin{aligned} 5. \text{ Intracardiac shunt, right to left (ml. per min.)} = \\ \text{Systemic blood flow (ml. per min.)} - \text{pulmonary artery blood flow (ml. per min.)} \\ \text{or,} \end{aligned}$$

$$\begin{aligned} 6. \text{ Intracardiac shunt, left to right (ml. per min.)} = \\ \text{Pulmonary artery blood flow (ml. per min.)} - \text{systemic blood flow (ml. per min.)} \end{aligned}$$

The effective pulmonary blood flow is the volume of mixed venous blood which, after its return to the right auricle, ultimately reaches the pulmonary capillaries. It is derived from the equation:

$$\begin{aligned} 7. \text{ Effective pulmonary blood flow (ml. per min.)} = \\ \frac{\text{O}_2 \text{ intake (ml. per min.)}}{\text{O}_2 \text{ content of pulmonary vein blood (vol. per cent)} - \text{O}_2 \text{ content of right auricular blood (vol. per cent)}} \times 100 \end{aligned}$$

Here again the assumption is made that pulmonary vein blood is fully oxygenated when blood cannot be obtained directly from that vessel.

In addition to the procedures already referred to, the standard exercise test described in a previous publication (3) was employed in these studies.



For presentation in this paper there have been chosen five cases of congenital heart disease. In three of these the anatomical diagnoses suggested by the physiological tests were confirmed at postmortem examination. In the remaining two cases the diagnoses provided by the physiological procedures were confirmed at operation. In the case

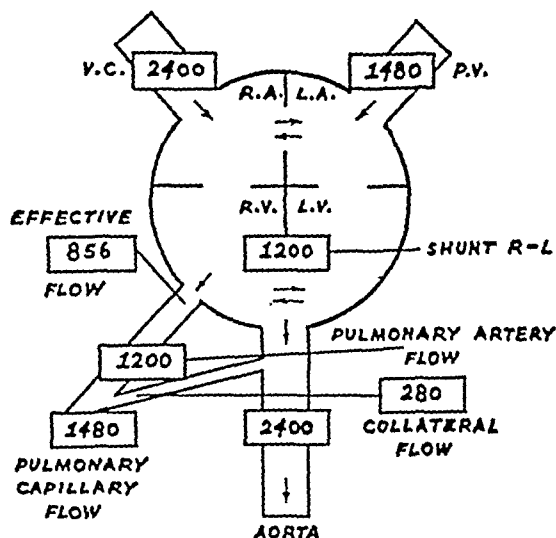


FIG. 1. illustrates the results from the physiological procedures in Case 1 (C. H.). Pulmonic stenosis is indicated by the volume of blood flow through the pulmonary artery which is 1200 cc. The presence of an interauricular septal defect is suggested by the gradient in  $O_2$  content between vena caval and auricular bloods (Table II). Similarly the presence of an interventricular septal defect is demonstrated by the gradient between auricular and ventricular bloods (Table II). The volume of the intracardiac shunt directed from right to left is probably made up of auricular and ventricular components. Since the effective blood flow to the lungs is 344 cc. less than the pulmonary artery flow there is evidence for left to right mixing of blood in the heart chambers.

histories only facts relevant to an understanding of the underlying physiological abnormalities will be listed.

#### CASE HISTORIES

*Case 1, C. H.,* was a fifteen year old youth first seen in March of 1946. His color at birth was momentarily blue but soon became normal. Although development was noticeably retarded, it was not until he was nineteen months old that

his parents learned he had heart disease. Following an attack of whooping cough, at two years of age, cyanosis became apparent with exercise but was not pronounced until the age of four. The course since that time had been one of progressive cyanosis and limited activity. Dyspnea had become extreme in the two years before he was seen here. At the time of admission his exercise tolerance was restricted to walking a distance of one hundred yards.

When examined he was a tall thin boy. Generalized cyanosis and clubbing of the fingers were extreme, with the cyanosis more evident in the upper extremities. A right dorsal scoliosis was accompanied by prominence of the left side of the chest anteriorly. A marked systolic thrill could be felt at a maximum in the third left intercostal space. The heart sounds were of good quality and the rhythm regular. Three distinct systolic murmurs were heard: a high-pitched whistling murmur at the base, a rough rumbling murmur over the precordium heard best in the third left intercostal space, and a systolic blow at the apex. Blood pressures in the arms and legs were equal at 125/100 mm. Hg. The remainder of the physical examination was not unusual.

Under the fluoroscope the heart was globular, rotated, and enlarged. The aorta was increased in outline and descended on the right side. The pulmonary conus was not prominent. In the left anterior oblique position the right ventricle bulged forward unduly. The pulmonary window was not clear. Lung markings were increased but active pulsations in the lung fields were not observed.

Electrocardiographic tracings with the standard leads revealed a normal sinus mechanism with sinus arrhythmia, and right deviation of the electrical axis. Routine laboratory tests resulted in a red blood cell count of 9.36 millions, a hemoglobin of 26 grams (Sahli), a hematocrit of 75 per cent, a non-protein nitrogen of 66 mgms. per cent, and a peripheral arterial  $O_2$  saturation of 63 per cent.

The clinical opinion was that the heart was rotated and that the aorta descended on the right. A single ventricle was suspected. Without definite evidence for pulmonary stenosis, operation could not be attempted. For this reason the patient was referred to the laboratory for physiological studies.

Results of physiological procedures: Some of the significant data obtained are illustrated in Figure 1. Pulmonary capillary blood flow calculated from the figures in Table I was 1480 cc.<sup>2</sup>, less than half the average normal cardiac index of 3000 cc. (4). Two catheterizations of the heart were performed (Table II). On March 14th the catheter introduced in the left arm entered the left side of the heart. The first auricular blood sample taken from the left border of the heart had an  $O_2$  saturation of 94 per cent, thereby indicating that it originated close to the entry of a pulmonary vein. With the catheter passing across the

<sup>2</sup> In this paper all figures for volume of blood flow are expressed in terms of cc. per minute per square meter of body surface.

upper border of the heart shadow and extending into the right pulmonary field, a second blood specimen was found to be 95 per cent saturated with oxygen. In view of the catheter position and the saturation of the blood sample it seemed probable that the pulmonary vein had been catheterized.

During the second catheterization through the right antecubital vein the catheter again entered the left side of the heart (Table II). A blood sample with 18.2 vols. per cent  $O_2$  was thought to originate from the right auricle. Specimens taken from various ventricular locations contained from 19.8 to 22.0 vols. per cent  $O_2$ . There was close corre-

TABLE 1

*Data Obtained from Equilibration Method for Determination of Pulmonary Capillary Flow*

NO.	DATE	SUBJ.	AGE	SEX	SURF. AREA $M^2$	$CO_2$ PROD. cc./min.	$CO_2$ CALC.	R. Q.	MIN. VOL. L/min.	$CO_2$ INCOMING BLOOD			$CO_2$ OUTGOING BLOOD			PULM. CAPILLARY FLOW L/min./ $M^2$
										%	Tension mm. Hg.	Vols. %	%	Tension mm. Hg.	Vols. %	
1	3/14/46	C. H.	15	M	1.6	229	155	1.2	9.27	4.22	30.3	27.5	2.81	20.1	21.0	1480
2	5/20/46	R. A.	22	M	1.5	157	127	1.0	6.93	3.84	27.5	27.2	2.84	20.1	22.7	2390
3	11/25/46	J. S.	32	F	1.5	133		0.7	4.7	4.81	34.2	44.1	4.07	29.0	41.0	2860
5	1/27/47	W. G.	16	M	1.7	206		0.9	9.25	4.10	29.3	26.5	3.33	23.7	22.9	3280

spondence between the  $O_2$  content of blood taken from the outflow tract of the right ventricle and that of the femoral artery (Table II).

In the absence of a representative sample for mixed venous blood, a circumstance occasioned by mixing of arterial and venous blood between the auricles, formulae for the calculation of systemic and effective pulmonary blood flows could not be applied. However, if the  $O_2$  content of blood from the inferior cava were to be considered as approximating that of the returning mixed venous blood, a systemic blood flow of 2400 cc. and an effective pulmonary blood flow of 856 cc. could be derived by applying Formulae 4 and 7. Pulmonary artery blood flow utilizing the sample from the outflow tract of the right ventricle was 1200 cc., approximately half the normal cardiac output (4). The intra-cardiac shunt directed from right to left was 1200 cc. and the collateral circulation to the lung 280 cc. (Formula 3).

*Comment:* In spite of the many anomalies in this heart, there were found the necessary prerequisites for operation. Pulmonary capillary and pulmonary artery blood flows were reduced to some 1480 and 1200 cc. respectively, and of these amounts slightly more than half was venous blood. (The effective pulmonary blood flow was 856 cc.) (Table II) (Fig. 1).

The predominating direction of the intracardiac shunt, right to left, was similar to that found in the tetralogy of Fallot (1). Finally with the peripheral blood  $O_2$  saturation at 69 per cent, it appeared likely that the effective blood flow to the lungs could be increased by constructing a "ductus" at operation.

Much of the information concerning the character of the congenital defects in this case was secured preoperatively by observation of the catheter movements within the heart. In this fashion, rotation of the heart, abnormal entry of the venae cavae, a single auricle, and a single ventricle were all suspected. The  $O_2$  content of blood from the heart chambers provided additional evidence for the presence of septal defects. The  $O_2$  content of blood from the inferior vena cava was lower than any of the auricular blood samples, suggesting that admixture of oxygenated and venous blood was occurring in the auricles (Table II). The same conclusion was drawn from the range of  $O_2$  contents in blood from the ventricles. Close correspondence between the  $O_2$  contents of blood from the outflow tract of the right ventricle and the femoral artery indicated that there was a functional single ventricle (Table II).

The predominant direction of the intracardiac shunt was right to left but there was concomitant shunting of blood in a left to right direction. Evidence for the latter was the observed volume of effective pulmonary blood flow which was 400 cc. less than the pulmonary artery flow and 620 cc. less than the pulmonary capillary flow (Table II).

The calculated collateral blood flow to the lungs was less than would have been expected from findings at operation and necropsy. In this instance the discrepancy may have lain in an unduly large calculated volume for pulmonary artery blood flow. The possibility of error in the calculation of pulmonary artery blood flow, in the presence of large collateral circulation to the lung, has already been discussed (3).

Procurement of fully oxygenated blood from the pulmonary vein in this case eliminates the possibility of a lung factor as being responsible

TABLE II

Data Obtained from Catheterization of the Heart

DATE	SUBJ.	AGE	SEX	SURF. AREA, M <sup>2</sup>	O <sub>2</sub> CON- SUMED cc./min.	CO <sub>2</sub> PRO- DUCED cc./min.	R.Q.	MIN. VOL. L/M	B.M.R.	O <sub>2</sub> VOLUMES PER CENT					FEMORAL ARTERY O <sub>2</sub>			FLOWS/M <sup>3</sup>		EFFECTIVE FLOW cc./min./ M <sup>2</sup>	SHUNT cc./min./ M <sup>2</sup>		
										V.C.	R.A. L.A.	R.V.	P.A.	P.V.	Con- tent	Capacity	Satura- tion	P.A.	Sys- temic				
3/14/46	C.H.	15	M	1.6	154	240	1.9	12.8	-40		31.3				32.0*	23.3	33.6	69.3					
3/18/46					195	153	0.8	6.22	-25		18.2	19.8	22.0			22.9				1200	2100	856	1200
5/23/46	R.A.	22	M	1.5	157	157	1.0	6.93	-25		23.1	26.7			33.5	28.9	35.2	82.0	1590	1850	1035	260	
11/27/46	J.S.	32	F	1.5	141	126	0.9	5.04	-24		13.0	12.5	14.4	18.5	18.5	18.5	19.8	93.5	2290†	1710	1710	0	
1/18/47	M.B.	18	F	1.3	127	127	1.0	8.14	-28		12.5	12.5	15.4	30.5	16.4	16.4	32.4	51.2	615	2100	517	1785	
1/28/47	W.G.	16	M	1.7	235	203	0.8	10.2	- 5	16.4	17.3	19.2		29.7	24.3	31.2	78.5	1280	1709	1011	429		

\* Direct Determination

† Pulmonary Capillary flow determined directly

TABLE III

Results Obtained from the Standard Exercise Test

NO.	DATE	SUBJ.	AGE	SEX	SURF. AREA M <sup>2</sup>	VENTILATION	CO <sub>2</sub>			O <sub>2</sub>		R.Q.	ARTERIAL BLOOD				
							cc. per lit.	cc. per L.V.	cc. per lit.	cc. per lit.	cc. per L.V.		O <sub>2</sub>	CO <sub>2</sub>	Vol. %	Cap.	Sat. %
3.	11/29/46	J.S.	32	F	1.5	Liters/min./M <sup>2</sup>											
						Rest	3.36	84.5	25.2	94.0	25.9	0.83	Vol. %	18.5	41.0	19.8	93.5
						Exercise	8.05	236.0	29.2	359.0	44.4	0.66	Vol. %	17.4	40.5	19.8	83.2
4.	1/20/47	M.B.	18	F	1.3	Rest	7.15	111.0	15.5	150.0	20.9	0.75	Vol. %	16.4	29.0	32.1	51.2
						Exercise	11.1	157.0	14.3	176.0	16.0	0.81	Vol. %	11.6	36.0	32.1	37.2
5.	1/28/47	W.G.	16	M	1.7	Rest	5.87	116.0	19.9	135.0	23.0	0.85	Vol. %	21.3	28.6	31.2	78.5
						Exercise	8.25	134.0	16.3	107.0	13.0	1.25	Vol. %	2.0	42.5	31.2	6.4*

for the symptom of cyanosis at rest. This finding is in agreement with others previously reported from this laboratory. Consequently peripheral O<sub>2</sub> unsaturation and cyanosis in this case are the result of shunting of blood from the venous to the arterial side of the circulation.

Following upon the performance of the physiological tests, operation was performed on June 6, 1946. A good many collateral blood vessels were encountered in the mediastinum. One of the venae cavae and the azygos vein lay on the left side of the mediastinum. A large left pulmonary artery was dissected out but pulsations in it were minimal. An end-to-side anastomosis between the left subclavian and left main pulmonary arteries was effected. After closure of the chest the pulse weakened and the patient succumbed within an hour.

At postmortem examination the heart was greatly enlarged, dilated and completely rotated so that the right ventricle lay on the left side. The superior vena cava and the pulmonary veins entered the auricle to the left of the interatrial septum while the inferior cava entered on the right. In essence there was a single functioning auricle because of a patent foramen ovale and an auricular septal defect 3 cm. in diameter. The ventricular walls were hypertrophied. The pulmonary artery arose from a chamber which was an anterior projection of the left ventricle. A white scarlike tissue almost occluded the pulmonary conus 5 cm. below a normal pulmonary valve. The aorta, arising chiefly from the left ventricle, straddled a 4 cm. defect in the interventricular septum.

*Case 2, R. A.*, was a twenty-two year old man, referred to the laboratory for study in May of 1946. At birth his color was blue and had remained so ever since. He always had had shortness of breath intensified by exertion and relieved by the assumption of a squatting position. During his early years there were many brief syncopal attacks which diminished in number as he grew older and finally ceased by the time he was fifteen years old. Despite the limitations of his illness he successfully completed secondary schooling. Six weeks before entry a coughing spell was said to have resulted in a hemoptysis. When seen in this hospital his exercise tolerance was limited to walking a distance of one city block.

On physical examination he appeared frail and underdeveloped for his age. Cyanosis of the skin, mucous membranes, and nail beds, and clubbing of the digits were immediately apparent. The chief positive findings were in relation to the heart examination. The precordium appeared normal. Over the lower end of the sternum on the left a faint systolic thrill was palpated. The heart

sounds, rate and rhythm were all normal but a moderately harsh systolic murmur could be heard. Its maximum intensity was at the lower left border of the sternum. A diastolic murmur was not found. Blood pressures in the arms and legs were the same at 100/80 mm. Hg. In the abdomen the liver edge was felt  $1\frac{1}{2}$  fingers breadth below the costal margin. The remainder of the examination was uneventful.

Under the fluoroscope the heart was not enlarged. There was a sharp concavity in the region of the pulmonary conus in the anteroposterior position. In the left oblique position the pulmonary window was clear. Although there were no pulsations in the lung fields themselves, minimal pulsations were present in the right hilar area. The aorta descended on the left side.

Electrocardiographic tracings with standard leads showed a normal sinus mechanism and deviation of the electrical axis to the right. Laboratory tests revealed a red blood cell count of ten millions, a hematocrit of 84 per cent, a hemoglobin of 21.9 gms. (Sahli), a non-protein nitrogen of 65 mgms. per cent, and a peripheral arterial  $O_2$  saturation of 82 per cent.

The history and physical findings in this case were those of the tetralogy of Fallot. It was believed that physiological studies would confirm the diagnosis established clinically.

**Results of physiological studies:** Some of the significant data derived from the tests are portrayed in Figure 2. Pulmonary capillary blood flow was reduced to 2390 cc. (Table I). Results from the heart catheterization revealed the right ventricular blood to be 3.6 vols. per cent higher in  $O_2$  content than that of the auricle (Table II). Pulmonary artery and effective pulmonary blood flows were 1590 cc. and 1035 cc. respectively. The collateral blood flow to the lungs was 800 cc. (Formula 3), and the intracardiac shunt directed from right to left was 260 cc. (Table II). Systemic blood flow was 1850 cc.

*Comment:* The circulatory measurements pointed to the diagnosis of tetralogy of Fallot. The pulmonary artery blood flow, half the average normal, spoke for stenosis of the artery. The auriculo-ventricular difference in blood  $O_2$  content indicating mixing of blood between the ventricles suggested the presence of an interventricular septal defect.

An unusual feature of the results was the volume of the intracardiac shunt, lower than most previously observed in the tetralogy of Fallot (1). Despite the small calculated shunt there was extensive mixing of blood through the septal defect as indicated by the auriculo-ventricular  $O_2$  gradient. Although the overall direction of the intracardiac shunt was right to left, a comparison of effective with pulmonary artery blood flow indicated that there was left to right shunting as well (Table II).

The effective blood flow to the lungs was 555 cc. less than the pulmonary artery blood flow. Evidence for right to left shunting of blood was found in the peripheral arterial unsaturation of 82%. There was present then reciprocal mixing and shunting of blood between the two sides of the heart with a small right to left shunt predominating.

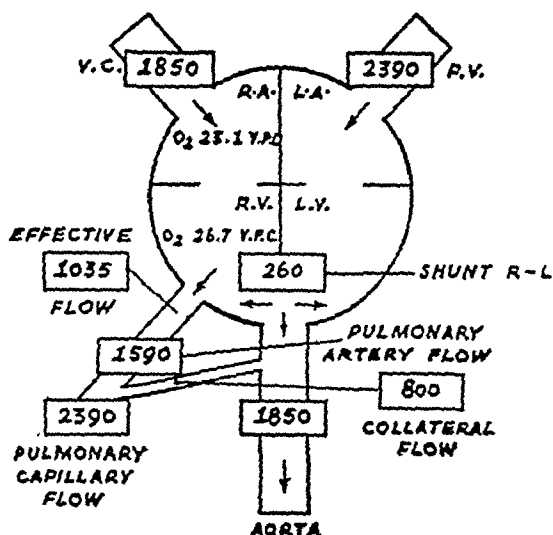


FIG. 2. illustrates the results from the physiological procedures in Case 2 (R.A.). Pulmonic stenosis is indicated by the volume of blood flow through the pulmonary artery. The presence of an interventricular septal defect is suggested by the difference in  $O_2$  content between auricular and ventricular bloods. The intracardiac shunt is small and directed toward the left. Evidence for left to right mixing of blood is the observation that the effective blood flow to the lungs is 555 cc. less than the pulmonary artery blood flow. These findings are typical for the tetralogy of Fallot.

The collateral circulation to the lungs was 800 cc. A third of the aortic output of 2650 cc., therefore, was diverted to the lungs through collateral blood vessels. The resulting decrease in peripheral blood flow to 1850 cc. may have had some bearing on the postoperative behavior of this patient. Upon the creation of an artificial ductus at operation there may have been a temporary further reduction in the volume of systemic blood flow below its original level. A sizable quantity of aortic blood, following the course of least resistance to the lung, was



made to short-circuit the periphery. Similar circulatory changes have been observed following the creation of an artificial ductus in dogs and in closure of the ductus in man during acute experiments by Eppinger, Burwell and Gross (5). The postoperative shocklike state shown by the patient under discussion may well have been part of the symptomatology of a markedly reduced peripheral blood flow and decreased venous return to the heart. Contributing to the picture was the blood loss at operation.

After completion of the special procedures, operation was performed on February 5, 1947. A moderate number of collateral blood vessels were seen in the mediastinum on the right side. The right pulmonary artery was relatively long, thereby confirming the preoperative fluoroscopic finding of hilar pulsations. The circumference of the artery was smaller than would have been expected for the patient's size. The pressure in it was low as measured with a saline manometer and pulsations were absent. An end-to-side anastomosis between the right subclavian and main right pulmonary artery was accomplished, after which a thrill was readily palpable. For two days postoperatively, the patient had persistent hypotension with the pressure never above 75/30 mm. Hg. He had anuria and moderate tachycardia. Toward the evening of the second day the patient became intensely cyanotic and soon expired.

At necropsy 400 cc. of bloody fluid were found in each pleural cavity. The heart was greatly enlarged with the right ventricular wall thicker than the left. The membranous portion of the interventricular septum had a moderate sized defect above which the aorta overrode both ventricles. Beyond the pulmonary conus, dilated in its lower half, there was stenosis at the pulmonary valve. The pulmonary artery widened beyond the valve and measured 2 cm. in circumference. The anatomical findings were those of the tetralogy of Fallot.

*Case 3, J. S.*, was a thirty-two year old married female first seen in November of 1946. The onset of symptoms associated with the present illness occurred during her first and only pregnancy. In 1941, in the fourth month of pregnancy, she had experienced undue fatigue and shortness of breath, both of which became more marked as pregnancy progressed. A physical examination in the seventh month revealed signs thought to be characteristic of a patent ductus arteriosus. The remarkable aspect of these findings was their discovery for the first time in

1941 despite many previous examinations. The patient's pregnancy was completed in a normal fashion. Since that time she had experienced overwhelming fatigue and breathlessness on slight exertion. There had never been cyanosis, orthopnea, or ankle edema. Her symptoms had forced her to remain in bed a good part of the day, but it was suspected that part of her disability was due to worry about her heart.

When examined she was well nourished and developed. The chief physical findings centered about the heart where a faint diffuse systolic heave was seen anteriorly over the left chest. A continuous thrill with systolic accentuation could be felt over the precordium. At the apex the heart sounds were normal in quality and the rhythm was regular. The predominating sound on auscultation was a loud continuous machinery-like murmur, accentuated during systole, heard maximally in the second left intercostal space, and audible over most of the left chest anteriorly and posteriorly. A faint systolic murmur was heard in the apical area. The blood pressure was 115/65 mm. Hg. in both arms. The remainder of the physical examination was not remarkable.

An antero-posterior x-ray view of the chest revealed the cardiac silhouette to be normal in size and contour. Under the fluoroscope the heart contracted vigorously and the apex excursions were wide. There were no abnormalities of the pulmonic conus or aortic knob. Pulsations were not seen in the lung fields.

Electrocardiographic tracings taken with the standard leads and chest leads were within normal limits. A stethogram portrayed a murmur with maximum intensity early in systole, which continued through the second sound and the first half of diastole.

Because of the discovery of a characteristic ductus murmur for the first time during pregnancy and relatively late in life, as well as a disproportion between symptoms and physical findings, this woman was referred to the laboratory for further study.

Results of physiological studies: Some of the findings are illustrated in Figure 3. Results from the heart catheterization may be seen in Table II. The variation in  $O_2$  content between auricular and ventricular blood was considered to be within the limits of error inherent in the catheterization technique (6). In calculating systemic blood flow, right auricular blood  $O_2$  content was employed to arrive at a flow of 1710 cc. Formula 4 (Table II). This was some 1000 cc. below the average normal cardiac index (4). Systemic and effective pulmonary blood flows were equal in amount (Table II). In the absence of evidence for intracardiac shunting of blood it was assumed that pulmonary artery blood flow was equal to the systemic flow.

Pulmonary capillary blood flow determined by the  $CO_2$  equilibration technique was 2860 cc. (Table I). Since Eppinger, Burwell and Gross

(5) have shown that complete mixture of arterial and venous blood in the smaller branches of the pulmonary artery occurs in patent ductus arteriosus, it was also possible to determine pulmonary capillary blood flow by catheterization of the pulmonary artery in the case under discussion. Employing the oxygen content of blood from the pulmonary

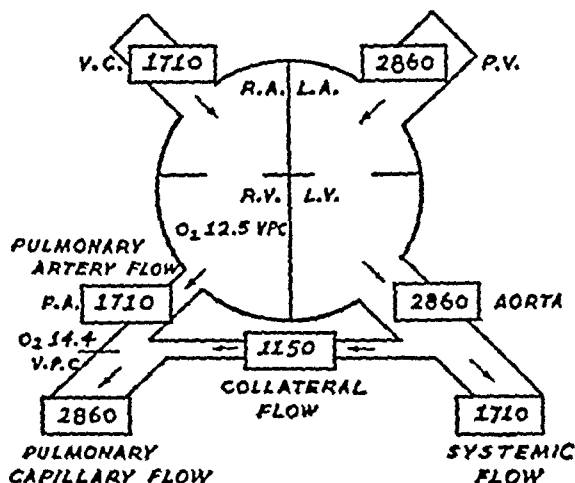


FIG. 3. illustrates the results from the physiological procedures in Case 3 (J. S.). Pulmonary capillary blood flow exceeds the pulmonary artery blood flow by 1150 cc., indicating that there is a large collateral circulation to the lungs via the ductus. The gradient in  $O_2$  content between ventricular and pulmonary arterial bloods demonstrates that oxygenated blood is entering the pulmonary artery through the ductus. Despite an aortic output of 2860 cc. the systemic blood flow is only 1710 cc. This reduction in systemic blood flow is probably secondary to shunting of blood away from the aorta.

artery (Table II), the pulmonary capillary flow was found to be 2290 cc. Thus there was fairly close agreement between capillary blood flows determined by the  $CO_2$  equilibration method and the catheterization technique (Tables I and II). The collateral blood flow to the lung calculated with Formula 3 was 1150 cc.

During catheterization of the heart the blood pressure in the right ventricle recorded with a Hamilton manometer was 33/10 mm. Hg. The pressure in the femoral artery was 150/90 mm. Hg., with a calculated mean pressure of 115 mm. Hg. This finding of an elevation in the

mean pressure (normal 90 mm. Hg) is contrary to the usual experience in large patent ductus where the pulse pressure is increased over the normal but the mean pressure reduced because of a lowered diastolic pressure (5).

Performance of the exercise test (Table III) resulted in a marked rise in the ratio,  $O_2$  consumed per liter of ventilation. From this it was inferred that the effective blood flow to the lung increased normally with exercise.

*Comment:* The collateral blood flow to the lung through the ductus in this patient comprised 40 per cent of the left ventricular output and was responsible for the fact that the output of that ventricle was 1.5 times that of the right. Despite a relatively normal left ventricular output of 2860 cc., the volume of the peripheral circulation was only 1710 cc. A similarly decreased peripheral blood flow was observed by Eppinger, Burwell and Gross following the creation of an artificial ductus in dogs and before closure of a patent ductus in man (5). It was reasoned, therefore, that the reduced systemic blood flow observed in this patient was secondary to a large collateral flow to the lung contributed by the ductus.

It is interesting that the  $O_2$  content of pulmonary artery blood was 1.9 vols. per cent higher than that of the right ventricle (Table II, Fig. 3). A gradient of this magnitude can be explained by admixture of oxygenated blood from the ductus with the mixed venous blood from the right ventricle. This finding is in agreement with the report of Eppinger, Burwell and Gross (5), which demonstrated that arterial blood is distributed throughout the pulmonary arterial system in patent ductus arteriosus.

The data presented thus far have described left to right extracardiac shunting of blood taking place via the ductus. However, the resting peripheral arterial  $O_2$  saturation of 92 per cent and the post-exercise saturation of 88 per cent (Table III) suggest the simultaneous occurrence of right to left shunting of blood. It hardly seems likely that there could have been reciprocal shunting through the ductus in view of the existing pressure gradient between the aorta and pulmonary artery. The similarity in the  $O_2$  content of right auricular and ventricular bloods did not indicate that intracardiac mixture of venous and arterial blood was taking place. To explain peripheral  $O_2$  unsaturation

in this case there are the remaining possibilities of deficient oxygenation or arteriovenous shunting in the lung per se.

Following completion of the special tests, the patient was operated on. After the left pleural cavity had been entered, a thrill could be felt over the pulmonary artery. A large patent ductus was found. Pressure on the ductus abolished the thrill. The ductus was then ligated. At the end of the procedure the thrill was absent. The postoperating course was uneventful, and a final physical examination before discharge from the hospital uncovered no residual heart disease.

*Case 4*, M. B., was a seventeen year old female referred to the laboratory for study in January of 1947. As far as her parents knew, she was normal at birth. At the age of one year, during an attack of pneumonia, the presence of congenital heart disease was made known to the parents. Cyanosis of the lips and face, as well as clubbing of the fingers and toes, became apparent at the age of two years and had been slowly progressive ever since. When seven years old the child had a brief period of unconsciousness followed by weakness of the left foot, which gradually improved. In recent years there had been many spontaneous spells of weakness, shortness of breath, palpitation of the heart, and intensification of cyanosis. Exercise tolerance was severely limited but varied with weather conditions. Several venesections performed during 1946 were followed in 24 hours by definite improvement in all her complaints.

When examined she was a poorly nourished underdeveloped girl. There was marked cyanosis of the face, lips, and nail beds, as well as clubbing of fingers and toes. Her posture was poor because of a kyphoscoliosis involving the lower thoracic and upper lumbar spines. Positive findings were confined chiefly to the heart. The heart did not seem enlarged and a thrill was not present. The sounds were of good quality with the rhythm regular, but a loud high-pitched blowing systolic murmur was heard over the entire precordium. It was at a maximum in the second left intercostal space. A diastolic murmur was not audible. The blood pressure in the arms was 130/100 mm. Hg. The remainder of the examination was not unusual.

Under the fluoroscope the heart was not enlarged. The aorta descended on the left. The pulmonary conus was not full in any projection. However, the pulmonary artery was prominent in the anteroposterior view and plainly crossed the aortic window in the left anterior-oblique position. Pulsations were not seen in the lung fields.

Electrocardiographic tracings with the standard leads revealed a deviation of the electrical axis to the right, and right ventricular hypertrophy with the precordial leads. Routine laboratory examinations resulted in a red blood cell count of 10.1 millions, a hemoglobin of 21.5 grams (Sahli), a hematocrit of 77 per cent, a non-protein nitrogen of 53 mgms. per cent, and a peripheral arterial O<sub>2</sub> saturation of 72 per cent.

Because of the large pulmonary artery seen at fluoroscopy, it was not clear whether the cyanosis was part of the symptomatology of the tetralogy of Fallot or due to some other abnormality. For this reason the patient was referred to the laboratory for further study.

Results of physiological tests: Some of the significant data derived from the tests are portrayed in Figure 4. Pulmonary capillary flow was

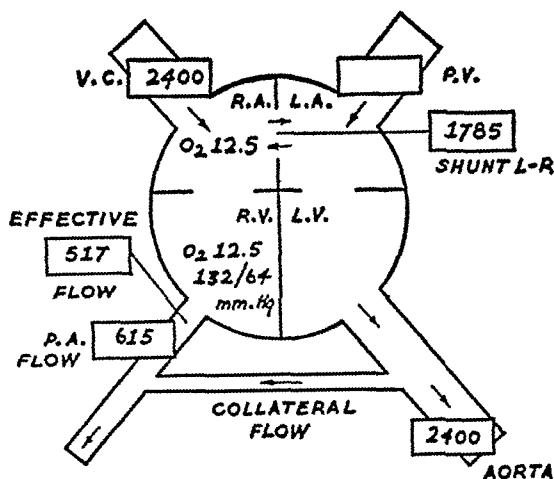


FIG. 4. illustrates the results from the physiological procedures in Case 4 (M. B.). Pulmonic stenosis is indicated by the volume of blood flow through the pulmonary artery which is 615 cc. Absence of a ventricular septal defect is suggested by the identical oxygen content of auricular and ventricular bloods. Presence of an auricular septal defect might have been revealed by the oxygen content of vena caval blood. The intracardiac shunt is predominantly right to left. Very little mixing occurs in the auricles because the effective pulmonary blood flow is only 100 cc. less than the pulmonary artery blood flow.

not measured in this individual. As a result the collateral blood flow to the lungs and aortic output could not be determined. The  $O_2$  contents of right auricular and ventricular blood obtained by catheterization were identical (Table II). These observations were in contrast to the findings in Cases 1 and 2 where a significant  $O_2$  gradient between the two chambers was encountered. Because of this finding, it was assumed that there was no interventricular septal defect. In the presence of an interventricular septal defect the  $O_2$  content of blood from the ventricle would most likely have been higher than that of the

auricle (1, 2). An important omission during the heart catheterization was the failure to procure a blood sample from the vena cava which might have provided definite evidence for the presence of the persistent foramen ovale found at necropsy.

The  $O_2$  content of pulmonary artery blood was 3 volumes per cent higher than that of the right ventricle (Table II). For the reasons outlined below, the  $O_2$  content of right ventricular blood was employed in calculating pulmonary artery blood flow (Formula 2). The blood flow through the pulmonary artery was found to be 615 cc. This indication of a severe stenosis was in keeping with the conditions found at necropsy.

The systemic blood flow was 2400 cc. (Table II). There is the possibility that auricular blood was not truly representative of mixed venous blood and higher in  $O_2$  content because of interauricular mixture of venous and oxygenated blood. For this reason the calculated systemic blood flow might have been greater than the true flow. Calculation of the effective pulmonary blood flow, which was 517 cc., suffered from the same criticism. As a sequel, the volume of the intracardiac shunt might have been less than the 1785 cc. derived (Table II).

Supporting the diagnosis of pulmonary stenosis were the results from the exercise test (Table III). The ratio,  $O_2$  consumed per liter of ventilation, fell from a resting value of 20.9 to 16.0, and the  $CO_2$  ratio also declined. There was also a fall in the peripheral blood  $O_2$  saturation from a resting value of 51.2 per cent to 37.2 per cent. From the latter result it was inferred that venous blood with a lesser  $O_2$  content or in greater quantities was shunted to the left side during exercise.

*Comment:* One of the criteria for location of the site of the intracardiac shunt is the relative  $O_2$  content of the blood in the heart chambers. Auricular septal defects, as subsequent experience in this laboratory has shown, provide the same opportunity for mixing of venous and oxygenated blood as do ventricular septal defects. In dogs, Brannon, Weems and Warren (8) have also found the  $O_2$  content of auricular blood significantly higher than that of the venae cavae in auricular septal defects. In the case under discussion it could not have been said that an interauricular shunt was present without a vena cava blood sample. In the absence of a shunt, peripheral  $O_2$  unsaturation would have had to be explained on the basis of some interference with oxygen-

ation in the lungs. The conclusions drawn from such a line of reasoning would have been in conflict with evidence derived from the history, physical examination, and the sum total of the circulatory measurements.

During the catheterization the catheter tip was passed into the pulmonary artery, a surprising occurrence in view of the marked stenosis of the orifice of that vessel found at postmortem examination. The blood sample obtained from the pulmonary artery was nearly three volumes per cent higher in content of  $O_2$  than that from the right ventricle. (Table II). In the absence of a patent ductus arteriosus there seemed to be two ways to explain this difference in  $O_2$  content. One was that the catheter blood sample represented oxygenated blood drawn through the capillaries in retrograde fashion from the finer radicals of the pulmonary vein (9). The second was that oxygenated blood contributed by collaterals from systemic vessels to the pulmonary artery was sampled (10). In favor of the latter explanation was the fairly close correspondence between the pulmonary arterial blood  $O_2$  content of 15.4 vols. per cent and that of the femoral artery with 16.4 vols. per cent (Table II). To have employed the  $O_2$  content of pulmonary arterial blood to calculate pulmonary artery flow would have resulted in an exaggerated flow through that vessel.

The blood pressure in the right ventricle, recorded with the Hamilton manometer, was 132/64 mm. Hg (Fig. 4). The height of the systolic pressure, some 100 mm. above the normal (7), was in keeping with those found in pulmonary stenosis of the tetralogy of Fallot (1). In view of the postmortem findings, the value for systolic pressure obtained suggested that the elevation was a result solely of stenosis. In the tetralogy of Fallot and Eisenmenger's complex there are, in addition to pulmonic stenosis, the overriding aorta and the interventricular septal defect to explain the elevations in systolic pressures found (1, 2). The elevation of the diastolic pressure recorded in this case could not be explained logically.

Following performance of the special tests, operation was attempted on January 22, 1947. As the pectoral muscle was being incised, tachycardia was noticed by the anesthetist. Soon thereafter the pulse weakened, became unobtainable, and never recovered.

At postmortem examination, collateral vessels were found coursing



from intercostal arteries through pleural adhesions to the lungs. A branch of the right internal mammary artery passed anomalously to the right middle lobe of the lung. In the heart, the foramen ovale was widely patent. The right ventricular wall was hypertrophied. An area of stenosis was present at the pulmonary valve where the leaflets fused and projected into the pulmonary artery to form a conelike exit. The opening into the artery was 2 mm. in diameter. Beyond the valve the artery was normal in size with the left branch somewhat dilated. Several supernumerary bronchial arteries arose from the aorta. In summary, then, this was not the tetralogy of Fallot but pulmonary stenosis associated with a patent foramen ovale.

*Case 5, W. G.*, was a sixteen year old boy referred to the laboratory for special studies in January of 1947. This patient was normal in appearance at birth and progressed satisfactorily until he was ready to enter school at five years of age. At that time, during a pre-school physical examination, a heart murmur was discovered. It was not until he was seven years old that blueness of the mucous membranes appeared. Simultaneously it was noticed that his tolerance for exercise was less than that of his playmates. In the space of a few years thereafter all of his symptoms, including cyanosis, dyspnea, and orthopnea, were intensified. His exercise tolerance soon became limited to walking a distance of several city blocks. At the age of twelve years he learned that squatting would relieve his shortness of breath. During the last two years he had appeared to lose ground rapidly. In the fall of 1946 a number of diagnostic tests, among them catheterization of the heart and angiocardiology, were performed at several other clinics with inconclusive results.

On physical examination performed in January of 1947 he was found to be a thin boy with intense diffuse cyanosis and marked clubbing of the fingers and toes. Percussion revealed the heart to be enlarged to the right and left. A diffuse systolic thrill was felt particularly well along the left sternal border. Heart sounds were of fair quality and the rhythm was regular. Over the entire precordium there was a loud rough systolic murmur heard maximally in the fourth left intercostal space. The murmur was transmitted toward the apex. The blood pressure in the arm was 96/70 mm. Hg. The remainder of the physical examination was not unusual.

Under the fluoroscope in the anteroposterior view the heart was enlarged to the left and right. There was a fulness in the region of the pulmonary conus. In the left antero-oblique position an unusual prominence, probably the upper portion of the right ventricle, was noted below the arch of the aorta. In the same view the left ventricle was much enlarged and the pulmonary window obliterated. Vascular markings were increased in the lung fields. Minimal expansile pulsations in the right hilar region were thought to be normal for an individual sixteen years old.

Electrocardiographic tracings with standard leads revealed right axis deviation and right ventricular hypertrophy with the chest leads. Routine laboratory examinations resulted in a red blood cell count of 8.8 millions, a hemoglobin of 23 grams (Sahli), a hematocrit of 74.5 per cent, a non-protein nitrogen of 48 mgms. per cent, and a peripheral arterial  $O_2$  saturation of 78 per cent. The atypical history of this patient and the finding of a large pulmonary conus at fluoroscopy called for further investigation before operation could be countenanced.

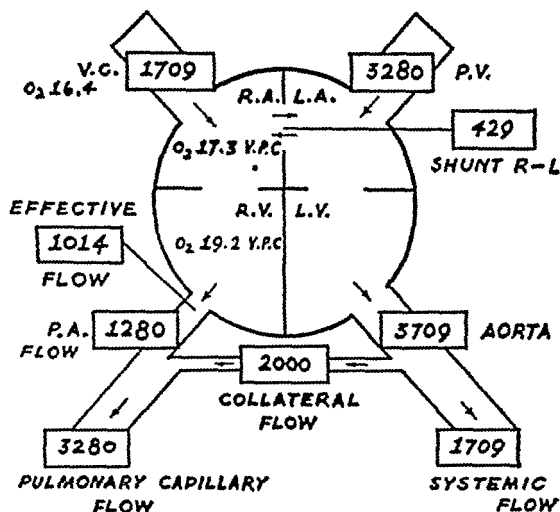


FIG. 5. illustrates the results from the physiological procedures in Case 5 (W G.). A large pulmonary conus was seen on fluoroscopy. However, pulmonic stenosis is indicated by the volume of blood flow through the pulmonary artery which is 1280 cc. The site of the intracardiac shunt is thought to be auricular because of the gradient in  $O_2$  content between vena caval and auricular bloods (Table II). The presence of a ventricular septal defect is less likely since the oxygen content of some auricular blood samples exceeds that of ventricular blood (Table II). A large collateral circulation to the lungs demonstrated here as 2000 cc. was found at operation.

Results of physiological tests: Some of the significant data are presented in Figure 5. Pulmonary capillary blood flow (Table I) was 3280 cc., a volume slightly above the average normal cardiac index (4). When the heart was catheterized the catheter entered the right side into what appeared to be a large auricular cavity. The pressures in this chamber, 25/11 mm. Hg, showed an increase in systolic and diastolic levels over normal auricular pressures (1). A series of blood samples taken from various auricular locations ranged from 17.3 to 21.4 volumes

per cent in  $O_2$  content (Table II). The  $O_2$  contents of blood samples from this chamber were at least one volume per cent higher than those of the vena cava (Table II). The experience here was somewhat like that in Case 1 of this report where a single auricle was later found at postmortem examination.

The right ventricle was located with difficulty in a downward and posterior location. It was only by observing continuous optical pressures during manipulation of the catheter that an area of high pressure (132/21 mm. Hg) was found. Pulmonary artery blood flow employing right ventricular blood  $O_2$  content was computed to be 1280 cc., less than half the normal cardiac index (Table II) (4). Collateral blood flow to the lungs (Formula 3) was 2000 cc., a volume in keeping with the great number of collateral vessels found at operation.

In calculating systemic and effective pulmonary flows, the difficulties experienced in Cases 1 and 4 in this paper were again encountered. Lacking a representative sample of mixed venous blood from the right auricle because of admixture of oxygenated blood in that chamber, the blood  $O_2$  content of the superior vena cava was again employed for these calculations. Volume flows of 1700 cc. for systemic blood flow and 1010 for effective pulmonary flow were thus obtained (Table II). The overall intracardiac shunt, probably interauricular in location, was 429 cc. and directed from right to left (Table II).

Performance of the exercise test yielded results compatible with the circulatory measurements already described. Both ratios,  $CO_2$  produced and  $O_2$  consumed per liter of ventilation, fell during exercise (Table III). This was confirmation for the diagnosis of pulmonary stenosis.

*Comment:* Only two of the anatomical abnormalities predicted by the circulatory measurements could be confirmed in this case because of the limited opportunity for exploration at operation. A large collateral circulation, thought to be about 2000 cc. preoperatively, was found. Reduced pulmonary artery blood flow was confirmed by the finding of a small pulmonary artery in which the pressure was visibly decreased.

There seemed to be little doubt that intracardiac mixture of venous and arterial blood was taking place. Right to left shunting of blood was indicated by the peripheral arterial  $O_2$  saturation of 78 per cent

(Table II). Probably there was left to right shunting as well because the effective blood flow to the lungs was less than the pulmonary artery flow. However, despite reciprocal mixing of blood, the predominant direction of the intracardiac shunt was from right to left. Once having established the presence of the shunt, a prerequisite for operation, its location was relatively unimportant from the standpoint of operative interference. Since the  $O_2$  content of right auricular blood exceeded that of the superior vena cava, by at least one volume per cent, it seemed that the site of the shunt was auricular (Table II).

Location of the right ventricle in this case was accomplished by the discovery of an area of elevated pressure within the heart, rather than by fluoroscopic observation of the catheter movements. The blood  $O_2$  content in this area indicated that the right ventricle rather than the left had been entered (Table II). Without this ventricular sample the pulmonary artery blood flow could not have been determined. The level of the systolic pressure in the right ventricle provided added confirmation for the diagnosis of pulmonary stenosis.

Following the performance of the tests described, operation was performed on February 1, 1947. It was evident, once the pleura was opened, that there were a great many collateral vessels in the mediastinum. An extremely small pulmonary artery was ultimately dissected out and pulsations in it were absent. Because of the small size of the artery an end-to-end anastomosis between right subclavian and main right pulmonary arteries was effected. When the anastomosis was completed a thrill could be felt and the pulmonary artery ballooned out as a large vessel.

#### SUMMARY

From a series of patients with congenital heart disease studied in this laboratory there were selected for presentation the histories of five individuals who required the application of physiological tests before surgery could be attempted. The cases were chosen because they illustrate some of the diagnostic problems amenable to solution by the procedures described. Operative or postmortem findings were available to validate allegations made on the basis of the tests performed.

In the first case, the diagnosis of pulmonary stenosis was not apparent from the physical examination and the fluoroscopic findings

were atypical. Catheterization of the right ventricle and pulmonary vein revealed a reduction in pulmonary artery blood flow. A decreased effective blood flow to the lungs was found by calculation. These findings, plus the diagnosis of an intracardiac shunt, indicated that the creation of an artificial ductus might be helpful (11). Catheterization of the heart revealed abnormal entry of a vena cava, an interauricular communication, and a virtual single ventricle.

The diagnosis of tetralogy of Fallot was confirmed by physiological studies in the second case. A large collateral circulation to the lungs predicted by the tests was found at operation. The possible relation of the large collateral circulation to the postoperative course of this patient was discussed.

In the third case, with classical signs of a persistent ductus arteriosus, certain elements of the history were unusual. By determination of pulmonary capillary blood flow and calculation of pulmonary artery blood flow, the volume flow through the ductus was found to be 1150 cc. At operation a large patent ductus was ligated.

In the fourth case, a large pulmonary artery seen at fluoroscopy created doubt as to the presence of pulmonic stenosis. Catheterization of the heart and performance of a standard exercise test revealed a reduction in pulmonary artery blood flow, a reduction in effective blood flow to the lungs, and the probable presence of an intracardiac shunt. Pulmonary stenosis was also indicated by an elevated right intraventricular pressure and a reduction in pulmonary artery pressure. These findings were sufficient to warrant operation. A patent foramen ovale and stenosis of the pulmonary valve were later found within the heart.

A prominent pulmonary conus, increased lung markings, and pulsations in the lung fields were all found at fluoroscopy in the fifth case. These findings seemed to contraindicate the presence of pulmonic stenosis. Performance of the exercise test and catheterization of the right ventricle indicated that there was pulmonary stenosis and reduced effective blood flow to the lungs. A large collateral circulation to the lungs, calculated from the physiological data, was found at operation. Reduced pulmonary artery blood flow was confirmed by the finding of a small pulmonary artery in which pulsations were absent.

We are indebted to Dr. James Bordley III for referring J.S. (Case III) to us for study.

## BIBLIOGRAPHY

1. BING, R. J., VANDAM, L. D., AND GRAY, F. D., JR., Physiological studies in congenital heart disease. II. Results of preoperative studies in patients with tetralogy of Fallot, *Bull. Johns Hopkins Hosp.* 80: 121, 1947.
2. BING, R. J., VANDAM, L. D., AND GRAY, F. D., JR., Physiological studies in congenital heart disease. III. Results in five cases of Eisenmenger's complex, *Bull. Johns Hopkins Hosp.* 80: 323, 1947.
3. BING, R. J., VANDAM, L. D., AND GRAY, F. D., JR., Physiological studies in congenital heart disease. I. Procedures, *Bull. Johns Hopkins Hosp.* 80: 107, 1947.
4. COURNAND, A., Measurement of cardiac output in man using right heart catheterization; Description of technique; Discussion of validity and of place in study of circulation, *Federation Proc.* 4: 207, 1945.
5. EPPINGER, E. C., BURWELL, C. S., AND GROSS, R. E., Effects of patent ductus arteriosus on circulation. *J. Clin. Investigation* 20: 127, 1941.
6. WARREN, J. V., STEAD, E. A., AND BRANNON, E. S., The cardiac output in man: A study of some of the errors in the method of right heart catheterization, *Am. J. Physiol.* 145: No. 4, 458, 1946.
7. BLOOMFIELD, R. A., LAUSON, H. D., COURNAND, A., BREED, E. S., AND RICHARDS, D. W., JR., Recording of right heart pressures in normal subjects and in patients with chronic pulmonary disease and various types of cardiovascular disease, *J. Clin. Investigation* 25: 639, 1946.
8. BRANNON, E. S., WEENS, H. S., AND WARREN, J. V., Atrial septal defect; Study of hemodynamics by technique of right heart catheterization, *Am. J. Med. Sci.* 210: 480, 1945.
9. DEXTER, L., Personal communication.
10. DUNCAN, G. W., Personal communication.
11. BLALOCK, A., AND TAUSSIG, H. B., Surgical treatment of malformations of the heart in which there is pulmonary stenosis or pulmonary atresia, *J.A.M.A.* 128: 189, 1945.



# THE ADMINISTRATION OF DI-ISOPROPYL FLUOROPHOSPHATE (DFP) TO MAN

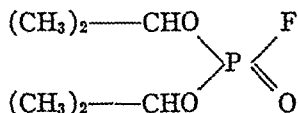
## I. EFFECT ON PLASMA AND ERYTHROCYTE CHOLINESTERASE; GENERAL SYSTEMIC EFFECTS; USE IN STUDY OF HEPATIC FUNCTION AND ERYTHROPOIESIS; AND SOME PROPERTIES OF PLASMA CHOLINESTERASE<sup>1</sup>

D. GROB, J. L. LILIENTHAL, JR., A. M. HARVEY, AND B. F. JONES

*From the Physiological Division, Department of Medicine, The Johns Hopkins University and Hospital, Baltimore, Maryland*

The alkyl esters of fluorophosphoric acid were discovered to be toxic inhalants by Lange and v. Krueger (23). Mackworth (27) demonstrated that the toxicity was due to the irreversible inhibition of cholinesterase (ChE) enzymes by these esters.

One member of this family of compounds has been subjected to particularly intensive study: *di-isopropyl fluorophosphate* (DFP).



Mazur and Bodansky (29) have demonstrated the extraordinary potency of DFP as an inhibitor of ChE both in vitro and in vivo and have shown in quantitative fashion that, in contrast to the transient effect of familiar anti-cholinesterase compounds, DFP inhibits irreversibly the ChE with which it comes in contact. The studies of Comroe (10), Modell (32), Horton (19), and their coworkers, and of Koelle and Gilman (21) indicated that the pharmacological effects of DFP administered to animals and man can be interpreted in terms of the ChE which had been destroyed.

The studies to be reported here and later record the observations made on certain effects of DFP noted during the course of its administration to more than 200 human subjects. Observations have been made on the following effects of DFP:

- a. On the blood (plasma and erythrocyte ChE activity levels and regenerative characteristics),

<sup>1</sup> Work performed under a contract between the Medical Division, Chemical Corps, U. S. Army, and the Johns Hopkins University.



- b. General systemic,
- c. On the gastro-intestinal tract (14),
- d. On the central nervous system (15),
- e. On the peripheral neuromuscular unit (18).

The present communication reports the observations made on (a) and (b), above, in normal subjects, in patients with myasthenia gravis, and in patients with liver disease, severe anemia, acute febrile and chronic debilitating diseases, and renal disease.

#### METHOD OF DETERMINATION OF CHOLINESTERASE ACTIVITY

Cholinesterase activity of plasma and red blood cells was determined by the method of Ammon (2) as modified by Mazur and Bodansky (29), in which 0.2 cc.

TABLE 1

*Plasma and red blood cell cholinesterase activity before the administration of DFP*

SUBJECTS	NO.	AVERAGE VOLUME OF CO <sub>2</sub> EVOLVED (C.MM.)		RANGE (C.MM. CO <sub>2</sub> )				STANDARD DEVIATION (C.MM. CO <sub>2</sub> )	
		By plasma	By red blood cells	Plasma		Red blood cells		Plasma	RBC
				Low	High	Low	High		
Normal and convalescent...	75	138	130	100	209	80	211	25	18
Myasthenic.....	12	139	117	98	182	88	158	12	14

of plasma, or 0.5 cc. of a 25 per cent red blood cell solution in sodium bicarbonate, is allowed to hydrolyze a standard solution of acetylcholine (0.015 M) in a bicarbonate buffer (0.04 M) and the liberated carbon dioxide measured at 37° C. in a Warburg manometer for 30 minutes. ChE activity was expressed in c.mm. as the average of the volumes of carbon dioxide evolved during three consecutive periods of 10 minutes. The volume of carbon dioxide evolved by a control solution from which plasma and red blood cell solution were withheld was subtracted from the experimental determination.

#### THE CHOLINESTERASE ACTIVITIES OF NORMAL AND MYASTHENIC SUBJECTS

The plasma and red blood cell ChE activity of 75 normal and convalescent subjects, and of 12 myasthenic subjects were studied prior to the administration of DFP (Table 1). In confirmation of Lucas and his co-workers (24) and others no difference was observed in these two groups. The day to day variation in 12 myasthenic subjects was studied (each morning, prior to the administration of any medication),

and the average daily variation was found to be 7 c.mm. of carbon dioxide (5 per cent) for both plasma and red blood cells. Over a period of weeks the variation was greater, sometimes 10 per cent or more. Differences in ChE activity between individuals could not be correlated with age, sex, weight, menstrual cycle, exercise, diet, or moderate fasting, in agreement with the observations of Vahlquist (39) on non-myasthenic subjects.

#### THE EFFECT OF DFP ON PLASMA AND RED BLOOD CELL CHOLINESTERASE ACTIVITY

DFP was administered intramuscularly in 0.1 or 0.2 per cent solution in peanut oil, in which solvent the compound remains active for at least a year. When DFP was administered intravascularly an aqueous solution (1 mg. per cc.) was prepared immediately before injection. This was necessary as the aqueous solution is much less stable, its half hydrolysis time being about 16 hours (1). The effect of the drug on plasma and red blood cell ChE activity was studied in 35 subjects (25 normal and 10 myasthenic). The doses given and the resulting depression of ChE activity are summarised in Table 2.

A single dose of DFP administered either intramuscularly (0.5 to 3.0 mg.) or intra-arterially (0.5 to 2.0 mg.) caused a marked depression of plasma ChE to between 35 and 5 per cent of original activity, and a much less marked depression of red blood cell ChE to between 95 and 65 per cent of original activity (Figure 1). The maximum depression of plasma ChE, to near zero activity, occurred within one hour after administration of the drug, while red blood cell ChE activity declined more slowly, maximum depression occurring 24 hours after administration (Figure 2). The daily intramuscular administration of 0.5 to 2.3 mg. of DFP caused an immediate and sustained fall in plasma ChE to between 20 and 5 per cent of original activity, and a slower progressive decline of red blood cell ChE at an initial rate of 5 to 10 per cent per mg. of DFP per day (Table 2 and Figure 3).

#### THE RETURN OF CHOLINESTERASE ACTIVITY AFTER THE CESSATION OF DFP ADMINISTRATION

##### *1. In Normal and Myasthenic Subjects*

Following cessation of the administration of DFP the plasma ChE activity began to increase within four hours, and the red cell ChE activ-

TABLE 2

*The effect of daily administration of DFP in oil on plasma and red blood cell cholinesterase activity*

NUMBER OF SUBJECTS	AVERAGE DOSE DFP PER DAY		NUMBER OF DAYS OF ADMINISTRATION	TOTAL DFP		LOWEST CHOLINESTERASE REACHED*—% OF CONTROL	
	mg.	mg./kg.		mg.	mg./kg.	Plasma	RBC
Normal and Convalescent (25)							
1	0.5	0.010	1	0.5	0.010	35%	99%
2	1.0	0.014	1	1.0	0.014	18	90
1	0.75	0.013	2	1.5	0.027	19	81
1	2.0	0.033	1	2.0	0.033	18	90
1	3.0	0.054	1	3.0	0.054	14	71
2	1.0	0.016	3	3.0	0.048	13	66
1	1.5	0.020	3	4.5	0.060	6	52
1	1.0	0.016	5	5.0	0.082	11	48
2	1.1	0.018	5	5.5	0.092	8	45
6	2.0	0.029	3	6.0	0.077	11	38
1	2.3	0.030	3	7.0	0.090	10	44
1	1.3	0.028	6	8.0	0.168	12	20
2	1.3	0.023	7	9.0	0.158	4	24
2	1.4	0.026	7	10.0	0.170	1	25
1	1.3	0.022	8	10.5	0.174	4	23
Average.....	1.38	0.021	3.8	5.35	0.085	11.3	50.1
Myasthenic (10)							
1	0.42	0.013	11	4.6	0.141	10%	16%
1	1.00	0.015	10	10.0	0.144	10	42
1	0.46	0.005	27	12.4	0.185	13	41
1	1.69	0.023	8	13.5	0.185	9	46
1	0.44	0.009	37	16.2	0.316	9	23
1	1.25	0.025	25	31.2	0.623	10	22
1	1.04	0.019	33	34.3	0.636	10	22
1	0.66	0.013	56	37.0	0.840	8	48
1	1.13	0.020	33	37.2	0.667	6	15
1	0.69	0.012	64	44.0	0.734	7	3
Average.....	0.80	0.014	32.6	23.88	0.445	9.2	27.8

\* 24 hours after the last dose of DFP.

ity within 24 to 48 hours and at a much slower rate (Figure 2). For plasma ChE the average rate of regeneration was 14 per cent of original activity during the first 24 hours, 9 per cent on the second day, 6 per

cent on the fifth day, 3 per cent on the eighth day and 2 per cent on the fifteenth day, while for red blood cell ChE the rate of regeneration was

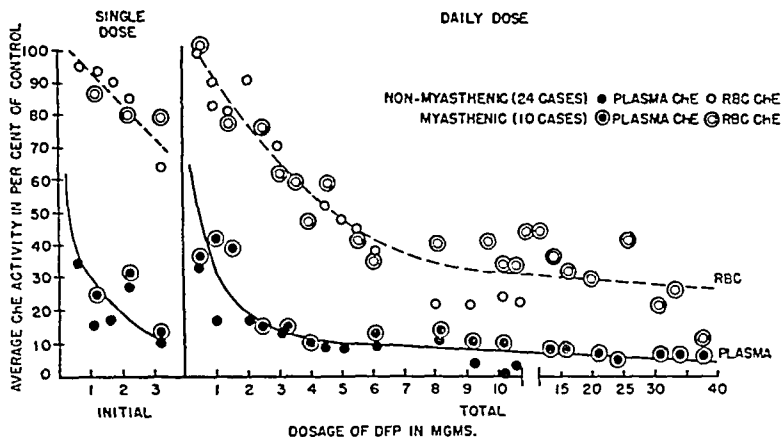


FIG. 1. THE EFFECT OF SINGLE AND DAILY INTRAMUSCULAR DOSES OF DFP IN OIL ON CHOLINESTERASE ACTIVITY (PLASMA AND RED BLOOD CELL)

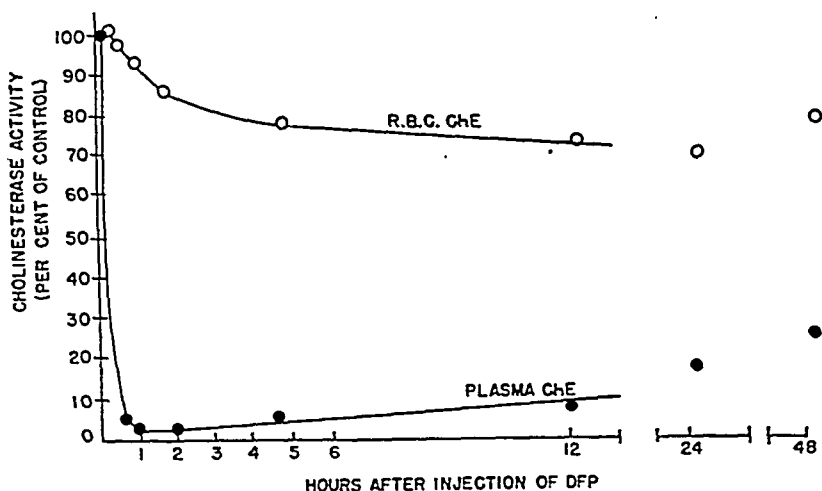


FIG. 2. THE EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF DFP IN OIL (2 MG.) ON CHOLINESTERASE ACTIVITY (PLASMA AND RED BLOOD CELL) (SUBJECT W. W.)

remarkably uniform at approximately one per cent of original activity per day (Figures 3 and 4).

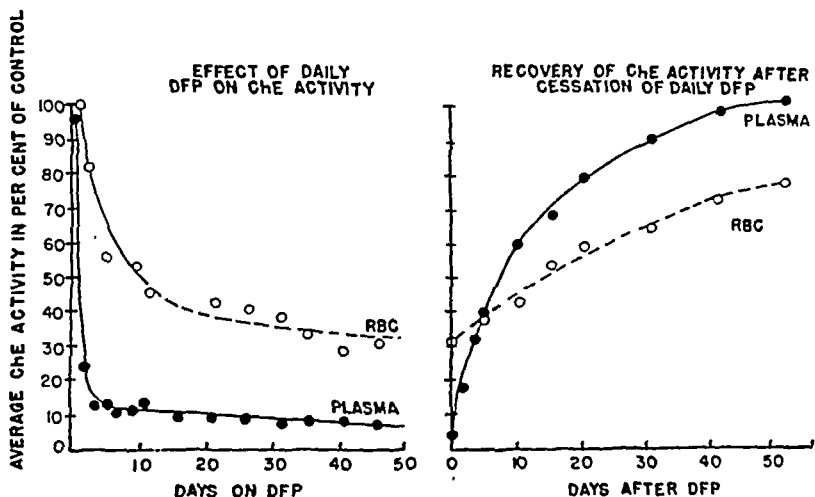


FIG. 3. The effect of the daily intramuscular administration of DFP in oil on cholinesterase activity (plasma and red blood cell), and recovery after cessation of DFP. Average values obtained in 35 subjects (25 normal or convalescent, and 10 myasthenic).

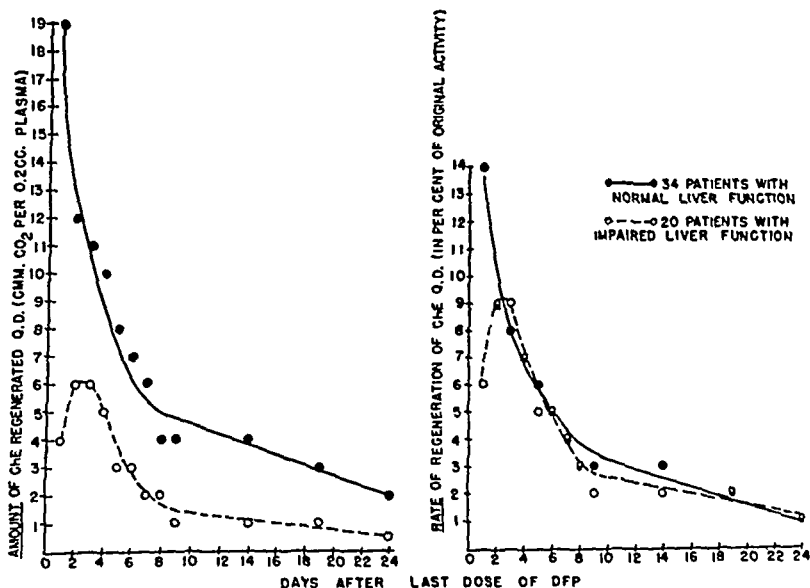


FIG. 4. The regeneration of plasma cholinesterase following cessation of the administration of DFP to normal subjects and to subjects with liver disease. (Average values represented.)

No subject who was followed for a sufficient length of time failed to show a return of the plasma and red blood cell ChE activity levels to those existing originally. Subjects who had previously received DFP and whose plasma and red blood cell ChE activity had returned completely to normal showed no evidence of sensitisation or of resistance to the drug. Finally, there was no significant difference between non-myasthenic and myasthenic subjects in the effect of DFP on plasma and red blood cell ChE activity, or on the rate of regeneration of ChE activity after the cessation of DFP administration.

## *2. Certain Regenerative and other Properties of Plasma Cholinesterase*

Mazur and Bodansky (29) have presented strong evidence that DFP destroys ChE irreversibly. The restoration of ChE activity after the cessation of DFP administration, therefore, probably represents the regeneration of new enzyme protein. This relationship has provided an opportunity to study certain factors which govern the formation of ChE enzymes in man.

The rate of regeneration of plasma ChE in man after its depression by DFP was found to resemble the rate of regeneration of serum albumin in experimental animals which have been depleted by plasmaphoresis (20, 37). This indirect analogy suggested that a study of plasma ChE activity and regeneration in patients with disturbances of protein metabolism might provide information regarding the site of production and properties of this enzyme protein. Such studies in patients with hepatic disease, severe anemia, acute febrile and chronic debilitating diseases, renal disease, hemoconcentration, and serous transudates and exudates are described below.

a. *Hepatic disease.* The study of 80 patients with various disorders of the liver and of 75 normal and convalescent subjects revealed that 90 per cent of the patients with impaired liver function had a reduction in the level of plasma ChE activity below the normal minimum of 100 c.mm. of carbon dioxide. In general, the degree of depression of plasma ChE activity paralleled the severity of liver damage as reflected by a variety of hepatic function tests (Table 3, B-E). In contrast to the plasma ChE, the red blood cell ChE activity was not impaired. It is of some interest that the reduced plasma ChE activity in these patients was not accompanied by any recognizable cholinergic symptoms.

The depression of plasma ChE activity was not as great in the

TABLE 3

*The influence of hepatic insufficiency, biliary obstruction, severe anemia, acute febrile and chronic debilitating diseases, nephrosis, and nephritis on plasma and red blood cell cholinesterase activity and regeneration. (Average values recorded)*

	A NORMAL CONTROLS	B	C	D	E	F	F <sup>1</sup>	G	H	I	J	K		
													Non obstructive hepatic insufficiency grouped in order of decreasing plasma ChE	Biliary obstruc- tion
Total number of cases*	75	23	26	24	7	11	4	21	13	12	9	6		
Plasma ChE activity (c mm CO <sub>2</sub> )														
Range	100-209	84-110	56-83	29-55	14-28	105-135	31-82	57-129	24-125	36-130	114-243	93-113		
Average	138	96	67	43	25	123	62	87	66	82	177	102		
RBC ChE (c mm CO <sub>2</sub> ) average	130	146	137	141	136	139	122	116	124	129	129	118		
Cephalin flocculation	0	1.5+	1.6+	2.1+	2.8+	0	0	0.6+	0.2+	1.0+		0		
Thymol turbidity (units)	3	8	11	12	16	5.7	5.9	9.5	9.6	11		5		
Prothrombin time (sec) after vit K admin	16	19	22	24	31	17	18	19		19	16	17		
Serum albumin (g %)	4.0	3.7	3.3	2.9	2.5	4.1	3.8	4.0	3.4	3.7	1.7	4.1		
Serum globulin (g %)	2.8	2.9	3.3	3.4	3.4	2.5	2.8	2.6	2.5	3.5	2.1	2.5		
Cholesterol (mg %)	220	192	156	179	93	248	299	144	136	155	581	207		
BSP retention (% after 30 min)	5	21	30	32	44	24	44	14	9	11	3	5		
Biirubin (mg %, total)	<0.8	3.2	4.6	10.0	21.4	9.0	12.6	2.4	1.1	1.1	<0.8	<0.8		
Alkaline phosphatase activity (Bod units)	4	5.6	6.2	10.8	6.3	17.7	23.7	4.1	3.1	5.1	3.9	2.9		
Amount of cholinesterase (c mm. CO <sub>2</sub> per 0.2 cc. plasma) regenerated daily during week after administration of DFP (15 mg. I.M. q.d. for 3 days)														
Number of cases	35	7	7	6	0	3	3	5	3	8	3	2		
Plasma	10	4.9	4.6	3.0		9.3	3.7	6.1	5.8		9.5	9.0		
RBC	1.3	1.2	1.4	1.8		1.6	1.7	1.4			1.0	1.0		

\* Description of cases A Normal and convalescent subjects B. Infectious hepatitis, 10; early cirrhosis, 7; metastatic carcinoma, 2; brucellosis, 1; lymphosarcoma, 1; Hodgkin's disease, 1; undiagnosed, 1. C. Cirrhosis, 20; leukemia, 1; lymphopathia venereum, 1; lymphosarcoma, 1; metastatic carcinoma, 1; undiagnosed, 2. D. Cirrhosis, 15; metastatic carcinoma, 5; sarcoid, 1; acute hepatic necrosis (terminal), 1; malaria, 1; undiagnosed, 1. E. Cirrhosis (terminal), 4; lymphoma (terminal), 3. F. Carcinoma, 5; biliary stone, 6. Average duration, 3.7 weeks. F. Carcinoma, 3; biliary stone, 1. Average duration, 11.5 weeks G. Post hemorrhagic, 4; pernicious anemia, 3; leukemia, 3; congenital hemolytic anemia, 2; sprue, 1; Banti's, 1; Felty's, 1; carcinomatosis, 1; thrombocytopenic purpura, 1; sickle cell anemia, 1; undiagnosed, 3. Average hematocrit, 22%. H. Pneumonia, 3; empyema, 2; tuberculous pleural effusion, 2, tuberculous pericarditis, 2, rheumatic fever, 2, gonococcal arthritis, 1; subacute bacterial endocarditis, 1. I. Lung abscess, 3, bronchiectasis, 2; lupus erythematosus, 2; pulmonary tuberculosis, 2; tuberculous peritonitis, 1; sarcoidosis, 1; eumatoid arthritis, 1. K. Average NPN, 110 mg %

TABLE 4  
*Changes in the plasma cholinesterase activity of patients with liver disease, anemia, and acute febrile illnesses. (Average values recorded)*

Number of Cases	A EFFECT OF PROGRESSION OF HEPATIC INSUFFICIENCY		B EFFECT OF IMPROVEMENT OF HEPATIC FUNCTION		C EFFECT OF RELIEF OF BILIARY OBSTRUCTION IN TWO PATIENTS WITH LOW PLASMA CHOLINESTERASE ACTIVITY		D EFFECT OF RECOVERY FROM RELAPSE OF FERNICIOUS ANEMIA		E EFFECT OF RECOVERY FROM ACUTE FEBRILE ILLNESSES	
	9		7		2		3			
Description	(3) Laennec's cirrhosis (2 terminal) (2) Lymphoma (terminal) (2) Lymphopatheic venous thrombosis (1) Severe anemia (1) Metastatic carcinoma		(3) Infectious hepatitis (1) Cirrhosis (1) Pernicious anemia (1) Lymphopatheic venous thrombosis (1) Malaria		(1) Carcinoma (1) Biliary stone		After 3 wks of folic acid or liver extract therapy		(3) Pneumonia (2) Empyema (2) The pericarditis (1) The pleural effusion	
	On admis sion to hospital	3 wks later	At height of illness	After im- provement (6 wks later)	During biliary obstruction	6 Weeks after relief of obstruction	16	35	During acute illness	2 weeks later
Cholinesterase Activity (c.mm. CO <sub>2</sub> )							Av. M C.V			
Plasma range	40-84	21-45	64-93	103-179	31-40	50-57	64-100	103-130	24-74	84-125
Plasma average.	60	30	70	131	35	54	73	120	53	108
RBC average	117	125	131	131	139	112	76	152	118	123
Cephalin flocculation	1.8+	2.5+	2.5+	0.2+	0	0	1.5+	0.5+	0.2+	0
Thymol turbidity	15	16	15	6	6	3	10	7.5	6	4
HSP retention (% after 30 min )	35	45	24	10	40	11	11	7	9	4
Bilirubin (mg.%, total)	11.6	18.6	3.7	1.0	8.8	2.2	2.7	1.0	1.0	<0.8
Alkaline phosphatase activity (Rodansky units)	7	7	6	5	14	5	4	4	3	3



patients with infectious hepatitis as in those with other diseases of the liver having a comparable degree of damage as judged by the functional tests. This difference may perhaps be related to the shorter duration of hepatic disease in this group.

Progressive impairment of liver function was accompanied by a continued fall in plasma ChE activity, which reached very low levels in patients who died in hepatic insufficiency (Table 4, A). Conversely, those patients who exhibited evidence of improvement in liver function showed a slow increase in plasma ChE activity, which reached normal levels in patients who made a complete recovery (Table 4, B).

In addition, 15 patients with jaundice secondary to obstruction of the biliary tract were studied. As judged by the usual tests their hepatic function was unimpaired. Eleven of these patients had normal plasma ChE activity (Table 3, F), but four, who had had biliary obstruction of long duration, showed a significant reduction in the activity of their plasma ChE (Table 3, F<sup>1</sup>). In two of the latter group relief of biliary obstruction was effected, and with the waning of their jaundice the plasma ChE levels began to rise very slowly (Table 4, C).

The regeneration of plasma and red blood cell ChE was studied in the patients of each group (Table 3) by administering 1.5 mg. of DFP daily for three days and then measuring the daily rise in esterase during the following week. There was a rough parallelism between the degree of depression of plasma ChE existing before the administration of DFP and the amount of ChE regenerated after destruction by DFP (Table 3 and Figure 4). In addition, there was a direct relationship between the degree of hepatic damage and the regenerative capacity which was even more consistent than the relationship between the degree of hepatic damage and the plasma ChE activity before the administration of DFP. A distinction must be made between the *amount* of plasma ChE regenerated daily, which was considerably reduced, and the *rate* of regeneration of plasma ChE (expressed in per cent of activity before the administration of DFP), which was closer to normal. Neither the amount nor the rate of regeneration of red blood cell ChE was altered significantly in these patients.

These observations suggest that the reduction in plasma ChE activity in patients with hepatic damage reflects an impairment in the mechanism for production of this enzyme protein. The possibility exists

that this reduction in ChE activity may have resulted from increased inhibition or destruction of the enzyme, but there was no evidence to support this possibility. The incubation of plasma from patients with liver disease with normal plasma yielded a mixture whose ChE activity was the mean of those of the original plasmas. The dialysis of the plasma of five patients with liver disease against running tap water for 24 hours resulted in only a slight decrease in the ChE activity, from an average of 48 c.mm. of carbon dioxide to 39 c.mm., while the protein which was precipitated during the dialysis was found to possess no ChE activity when it was redissolved. These observations provide evidence against the presence of free or dialysable inhibitors of ChE in the plasma of patients with liver disease.

b. *Severe anemia, acute febrile and chronic debilitating diseases.* Depression of plasma ChE activity and of plasma ChE regeneration after the administration of DFP were not restricted to patients with out-spoken diseases of the liver. Lesser, but significant, degrees of depression of both were encountered in patients with severe anemia (especially macrocytic anemia), and with a variety of acute febrile and chronic debilitating diseases (Table 3, G-I). The depression of plasma ChE regeneration after DFP in these patients was in general proportional to the existing depression of plasma ChE activity. There was no abnormality of the red blood cell ChE activity or regeneration in these patients, except for some cases of leukemia and of pernicious anemia in relapse, in which these too were depressed.

Many of these patients who had more marked depression of plasma ChE activity and regeneration also showed evidence of mild impairment of liver function, as reflected by the usual tests (Table 3, G-I). However, this was not invariable, and furthermore, the degree of reduction of both functions was usually more marked than the degree of liver impairment that was indicated by these tests. There was no observed relation of ChE activity to body temperature, bacterial invasion, leucocytosis, food intake, electrolyte balance, or inactivity. The addition of the plasma of these patients to normal plasma failed to demonstrate the presence of free inhibitors of ChE activity, and the dialysis of the plasma of these patients against running tap water for 24 hours failed to demonstrate the presence of dialysable inhibitors of ChE activity.

Recovery from the anemia or acute febrile illness resulted in a return

to normal of the plasma ChE activity (Table 4, D-E), and in diminution or disappearance of any signs of liver impairment. Improvement in chronic disease was accompanied by a more gradual increase in the plasma ChE activity.

c. *Renal disease.* Nine patients who were in the nephrotic stage of chronic glomerular nephritis with marked hypoalbuminemia and moderate hypoglobulinemia were found to have normal or elevated plasma ChE activity (Table 3, J). These patients, who had normal hepatic function by the ordinary tests, also had normal regeneration of plasma ChE after the administration of DFP. This is in contrast to the patients with liver disease, in whom reduction in the concentration of

TABLE 5

*The effect of hemoconcentration due to acute mercury poisoning on cholinesterase activity, concentration of serum proteins, and hematocrit (9 cases, average values recorded)*

TIME (HRS.) AFTER INGESTION OF BICHLORIDE OF MERCURY	CHOLINESTERASE ACTIVITY (C.MM. CO <sub>2</sub> )		CONC. OF SERUM PROTEINS (G.%)			HEMATOCRIT
	Plasma	Red blood cells	Total proteins	Albumin	Globulin	
1	190	136	8.2	5.5	2.7	45
2	190	140	8.2	5.5	2.7	45
24	140	138	6.4	4.4	2.0	40
48	132	132	6.3	4.3	2.0	40

serum albumin was accompanied by reduction in the activity and regeneration of plasma ChE (Table 3, B-E).

Six patients in the advanced stage of chronic glomerular nephritis had plasma ChE activities which are considered to be at the lower limits of normal. The regeneration of plasma ChE after its depression by DFP was approximately normal in two of the patients who were so studied. The red blood cell ChE activity and regeneration, the concentration of serum albumin and globulin, and the tests of liver function were normal in this group (Table 3, K).

d. *Hemoconcentration.* In patients with hemoconcentration, evidenced by an increase in plasma protein concentration and volume of packed erythrocytes, there was a proportional rise in plasma ChE activity (Table 5). There was no alteration in the ChE activity of the red blood cells. The hemoconcentration in the nine patients studied was

due to acute bichloride of mercury poisoning with loss of fluid and electrolytes into the gastrointestinal tract. All these patients received dimercaptopropanol (British Anti-Lewisite), but administration of this drug to seven normal individuals, resulted in no change in ChE activity, serum protein concentration, or volume of packed erythrocytes.

e. *Transudates, exudates, and urine.* The ChE activity of transudates, exudates, and urine was studied in 66 patients. Since addition of these fluids to normal plasma, or dialysis of the fluids, failed to demonstrate the presence of free or dialysable inhibitors of ChE, it has been assumed that the determined activity of the fluids was directly proportional to the amount of ChE which had diffused from the plasma. Contributions to the protein content of the fluids by the cells of the serous membranes and kidneys have, for the purpose of calculation, been assumed to be negligible.

The amount of ChE in transudates and exudates was the resultant of two functions: its level in the plasma and its diffusibility. Diffusion was in general proportional to, but slightly less than, the diffusion of serum albumin and globulin (Table 6). Transudates, and exudates with little protein, were practically devoid of ChE activity. Those exudates which contained larger amounts of protein had proportionately greater ChE activity. These relationships were independent of the membrane involved (peritoneal, pleural, pericardial, or synovial).

In marked contrast to the generally parallel diffusions of plasma proteins and ChE into exudates were the differences in their appearance in the urine of patients in the nephrotic stage of chronic glomerular nephritis, where the concentration of albumin usually exceeded that in the plasma, and the concentration of globulin approached that in the plasma, while the concentration of ChE never exceeded ten per cent of its plasma concentration (Table 6).

The urine of normal subjects was devoid of ChE activity. The urine of six patients with chronic nephritis, containing less than 0.3 g. per cent of protein, had little or no ChE activity (Table 6).

### 3. *The Regenerative Properties of Red Blood Cell Cholinesterase*

The average rate of regeneration of red blood cell ChE following its depression by DFP was found to be approximately 1.2 per cent of original activity per day (Figure 3). This figure is similar to the re-

placement rate of the red blood cells and indicates that the limiting factor in the regeneration of red blood cell ChE is the rate of replacement of the red blood cells themselves. There is some difference of

TABLE 6

*The diffusion of albumin, globulin, and cholinesterase from the plasma into transudates, exudates, and urine*

	DISEASE	BODY FLUID	NO. OF CASES	ALBUMIN CONCENTRATION* IN BODY FLUID (G. PER CENT)		GLOBULIN CONCENTRATION* IN BODY FLUID (G. PER CENT)		CHOLIN-ESTERASE ACTIVITY OF BODY FLUID (C.MM. CO <sub>2</sub> PER 0.2 CC.)		PER CENT DIFFUSION FROM PLASMA INTO BODY FLUID		
										conc. in body fluid		
										conc. in plasma		
				Range	Av.	Range	Av.	Range	Av.	× 100%		
										Albu- min	Glob- ulin	ChF
Transu- dates	Nephrosis	Edema	3	0.01-0.02	0.01	0.01-0.02	0.02	0-1	0.3	<1	<1	<1
Exudates	Nephrosis	Pleural	1		0.1		0.1		1.5	9	5	1
	Cirrhosis	Ascitic	6	0.1-0.4	0.3	0.1-0.8	0.4	1-6	3.8	11	11	6
				1		2.6		1.5		16	67	53
		Pleural	1		0.8		0.6		7	30	17	9
	Neoplastic	Ascitic	2	0.6-0.8	0.7	0.7-1.1	0.9	13-15	14	24	26	21
			Pleural	1		1.5		1.3		12	38	50
	Polysero- sitis	Ascitic	2	0.6-1.9	1.3	0.5-1.0	0.8	13-38	26	33	33	29
			Pleural	5	1.3-1.9	1.7	0.7-1.9	1.4	13-56	41	52	52
	Cardiac failure	Ascitic	1		0.1		0.1		1	4	2	1
				3	1.4-1.6	1.5	1.1-1.3	1.2	18-30	26	41	60
		Pleural	1		1.4		1.3		14	52	35	48
	Infectious	Ascitic		2	1.3-1.6	1.4	1.6-2.0	1.8	15-18	17	55	47
			1	0.4		0.2		7	17	7	12	
Pleural			8	1.7-3.7	2.7	0.8-2.5	1.8	16-70	44	76	66	67
			4	1.9-3.0	2.5	1.1-4.1	2.3	24-60	42	70	74	70
Pericar- dial Joint			4	2.2-3.8	3.0	0.9-2.5	2.2	47-91	69	75	77	76
Urine	Normal	Urine	3	0	0	0	0	0	0	0	0	0
	Nephritis	Urine	6	0.03-0.2	0.1	0.02-0.1	0.04	0-2	0.5	2	2	<1
	Nephrosis	Urine	11	0.4-4.0	2.3	0.2-2.6	1.3	0-26	6	155	61	4

\* Determined by fractional precipitation with neutral salt (Howe), except for the nephrotic urines, which were studied electrophoretically (Tiselius).

opinion regarding the normal replacement rate of circulating erythrocytes, but most of the experimental work (employing the method of selective agglutination of transfused erythrocytes and more recently radioactive and heavy isotopes) indicates a replacement rate of 0.8 to

1.2 per cent daily, corresponding to a life span for the red blood cell of 83 to 125 days (4, 7, 36, 40, 41). The average rate of regeneration of red blood cell ChE after its depression by DFP indicated a red blood cell replacement rate of 1.2 per cent daily, corresponding to a life span for the red blood cell of 83 days.

Additional evidence for the relationship between the regeneration rate of red blood cell ChE and the replacement rate of the red blood cells is furnished by a study of patients with altered red cell replacement rates. In patients with low reticulocyte counts there was a corre-

TABLE 7

*The relation between the rate of regeneration of red blood cell cholinesterase after cessation of DFP and the reticulocyte count*

NO. OF CASES	RETICULOCYTE COUNT (PER CENT)		DAILY RATE OF RISE OF ChE DURING FIRST WEEK AFTER CESSATION OF DFP (IN PER CENT OF CONTROL)			
	Range	Average	RBC ChE		Plasma ChE	
			Range	Average	Range	Average
13	0.2-1.0	0.7	0.2-1.9	0.8	5-13	7.1
24	1.1-2.0	1.4	0.7-1.7	1.2	6-11	7.6
6	2.1-3.0	2.6	2.3-2.9	2.6	5-12	7.6
1*		5.0		10.8		7.5
1†		10.8		13.0		9.5
1†		13.0		16.0		10.4
1†		13.6		17.0		10.5
1†		15.4		19.0		11.2

\* Case M. D., pernicious anemia in relapse under liver therapy, during reticulocytosis.

† Case B. C., pernicious anemia in relapse under folic acid therapy, during reticulocytosis. Daily values given.

sponding low rate of regeneration of red blood cell ChE after cessation of DFP administration; and, conversely, in those patients with increased reticulocyte counts there was a corresponding increase in the regeneration rate of red blood cell ChE (Table 7). Furthermore, the rate of rise of red blood cell ChE activity in a given patient varied with the number of reticulocytes in the peripheral blood. For example, in one patient with pernicious anemia who was treated with folic acid the maximum reticulocyte response of 15 per cent was accompanied by a 19 fold increase in the rate of regeneration of red blood cell ChE (Table 7). In contrast, there was only a slight increase above normal in the

rate of regeneration of plasma ChE. Control studies have indicated that folic acid (in the absence of reticulocytosis) does not alter the plasma or red blood cell ChE activity either *in vivo* or *in vitro*.

In some cases of pernicious anemia in relapse and of leukemia the ChE activity of the red blood cells was below normal. The reticulocytosis which followed specific therapy of the former was accompanied by an increase in the ChE activity of the red blood cells. Thus, in three cases of pernicious anemia in relapse whose red blood cell ChE activity averaged 76 c.mm. of CO<sub>2</sub> before treatment, the daily administration of folic acid or liver extract resulted in a marked reticulocytosis and a sharp increase in the red cell ChE activity, which reached an average of 152 c.mm. of CO<sub>2</sub> after three weeks of therapy (Table 4, D). Similar observations have been reported by Sabine (34) following the administration of liver extract to patients with untreated pernicious anemia.

Davis (11) has suggested that the mechanism of the anti-anemic action of folic acid and liver extract is concerned with its influence on ChE activity. It is true that an increase in red blood cell ChE activity usually accompanied the reticulocytosis that followed the institution of therapy of pernicious anemia, but this rise in activity was found to be not essential to the anti-anemic effect of folic acid or liver extract. For, in two patients with pernicious anemia a complete remission was induced by folic acid and by liver extract therapy in spite of the sustained suppression of plasma and red blood cell esterase activity by the daily administration of DFP.

#### THE SYMPTOMS THAT FOLLOWED THE ADMINISTRATION OF DFP

The symptoms that followed the daily intramuscular administration to 60 subjects (50 normal and 10 myasthenic) of 1.5 to 3 mg. of DFP for two to five days mimicked most of the muscarinic and nicotinic effects of cholinergic drugs, with the exception of peripheral vasodilatation and of fall in blood pressure (Table 8). In addition, there were striking symptoms referable to the central nervous system.

In patients receiving small daily doses of DEP (1.5 mg. daily or less), central nervous system manifestations were usually the first to appear, followed by gastro-intestinal symptoms. The character of these central nervous system symptoms is illustrated in the case report below, while the frequency of their occurrence may be seen in Table 8. Their

**TABLE 8**  
*Symptoms following the daily intra-muscular administration of DFP*

EFFECT OR ORGAN	SYMPTOM	NO. OF SUBJECTS (TOTAL: 10 NORMAL AND 10 MYASTHENIC)
1. Muscarinic a) Gastro-intestinal	1. anorexia-nausea 2. abdominal cramps 3. vomiting 4. diarrhea 5. cardiopasm 6. nausea after smoking	41 29 25 14 12 4 4
b) Sweat glands	increased sweating	10
c) Lacrymal glands	increased lacrymation	3
d) Salivary glands	increased salivation	2
e) Pupils	slight miosis	2
f) Ciliary body	difficulty of distant vision	2
g) Lungs	respiratory difficulty, suggestive of bronchoconstriction	2
h) Bladder	urinary frequency	2
i) Heart	slight bradycardia with premature contractions	1
2. Nicotinic Skeletal muscles	fasciculations (non-myasthenics only) increased strength (myasthenics only) decreased strength (non-myasthenics only) muscular cramps	14 7 10 4 1
3. Central nervous system	excessive dreaming insomnia jitteriness, restlessness, increased tension, emotional lability, tremulousness nightmares, frequently with talking in sleep headache increased libido fiddiness drowsiness paresthesias mental confusion visual hallucinations tremor pain in legs, sciatic distribution	49 33 29 29 17 8 6 6 5 3 2 1 1 1
4. Skin	urticaria (may have been sensitive to peanut oil)	2
5. No symptoms		6



intensity was proportional to the amount of DFP which the patients received. Accompanying electroencephalographic changes will be discussed in a subsequent report (15).

Those patients who received larger doses of DFP (more than 1.5 mg. daily) frequently developed gastro-intestinal symptoms first, followed by central nervous system symptoms. Anorexia was usually the first symptom to appear, followed by nausea, abdominal cramps, vomiting, and diarrhea as the degree of the DFP effect increased. Definite effects on other autonomic functions (sweating, lacrimation, salivation, miosis, ciliary spasm, bronchoconstriction, urinary frequency, and bradycardia) appeared much less frequently and usually only after larger doses of the drug.

The muscarine-like and central nervous system effects were the same in the normal and myasthenic subjects, while the nicotine-like effects were different. Muscular weakness and fasciculations occurred in some of the normal subjects after large doses of DFP, in contrast to the increased motor power, without fasciculations, that occurred in all the patients with myasthenia gravis.

The muscarinic and central nervous system symptoms disappeared within one to four days after the last dose of DFP. The nicotinic symptoms in non-myasthenic subjects disappeared within the same period. In myasthenic subjects who had received larger total doses of DFP (Table 2) the increased strength frequently persisted for as long as seven to ten days after the last dose of the drug.

The repeated administration of DFP resulted in an increasing effect of each dose, presumably because of the progressive irreversible inhibition of ChE. Repetition of the administration of DFP within three or four weeks of the last dose of the drug also resulted in an increased effect, presumably because the regeneration of ChE during this interval had not been complete.

Atropine given by any route had a marked inhibitory effect on the muscarinic symptoms due to DFP, a moderate inhibitory effect on the central nervous system symptoms, and no effect on the nicotinic symptoms. The administration of atropine was the best means of reducing the gastro-intestinal and central nervous system symptoms caused by DFP.

The following case report illustrates most of the manifestations that follow the administration of this drug.

A 56 year old white male subject was given daily intramuscular injections of DFP for three days. Twelve hours after the first injection of 3 mg. he began to have anorexia, mild nausea, and excessive dreaming. The content of the dream was that he was lost, deserted by his friends, and attacked by wild animals. On the following morning his plasma ChE was 16 per cent of normal activity and his red blood cell ChE 70 per cent. He was then given a second injection of DFP (2 mg.) and within two hours developed increased anorexia and nausea, restlessness, irritability, a feeling of tremulousness and tension, a dull headache and increased libido. That night he had insomnia and terrifying nightmares. The patient also had a nocturnal seminal emission, the first in many years. On the third day he was given another injection of 2 mg. of DFP. One hour later he developed marked anorexia, followed by nausea, vomiting, mild abdominal cramps, urgent defecation, sweating, and some generalized weakness. During the day he also had a sensation of tightness in the chest with a feeling of pressure on swallowing, suggestive of cardiospasm. The central nervous system symptoms became more marked, and he was also intermittently drowsy. That night the insomnia and nightmares were very troublesome, and the patient was heard to cry out frequently in his sleep. The following morning the plasma ChE was 10 per cent of original activity, and the red blood cell esterase 44 per cent. The symptoms diminished gradually and then disappeared during the next 48 hours.

THE RELATION OF SYMPTOMS DUE TO DFP TO THE  
CHOLINESTERASE ACTIVITY OF THE PLASMA  
AND RED BLOOD CELLS

Following the intramuscular administration of 1.5 mg. of DFP per day for five days, 2 mg. per day for four days, or 3 mg. per day for two days, the development of moderate symptoms was observed in almost all cases.

It became evident almost at once that the depression of plasma ChE activity bore no relation to the appearance of symptoms. A single dose of 1 to 2 mg. of DFP depressed the plasma ChE level almost to zero without the appearance of symptoms (Figure 2). There was, however, an apparent relationship between red blood cell ChE activity and the appearance of symptoms. When DFP was administered daily in doses of 1.5 to 3 mg. symptoms usually appeared when the red blood cell ChE level fell below 70 per cent of the original activity and became very troublesome when the level fell below 40 per cent. When, however, DFP was administered for long periods in smaller dosages (0.5 to 1.0 mg. each day), symptoms were absent or appeared transiently and then vanished although the red cell ChE activity fell below 25 per cent of original activity. Conversely, no matter how low the red blood cell

esterase had been depressed symptoms disappeared within 48 hours after DFP administration was stopped although the rise in red blood cell ChE activity during this time was negligible.

These observations indicate that the development of symptoms due to DFP bore no constant relation to the ChE activity of either the plasma or the red blood cells. The appearance of symptoms probably is related more directly to the activity level of ChE enzymes in the tissues of the effector organs themselves.

#### THE TOXICITY OF DFP

Ten patients with myasthenia gravis who received daily intramuscular injections of DFP for several weeks in total doses up to 0.84 mg. per kilo of body weight (Table 2) were studied by means of a number of examinations aimed at disclosing any general or specific toxicity other than the effects noted in Table 8. The administration of the drug produced no changes in pulse, temperature, respiratory rate, blood pressure, or body weight. No significant changes were noted in the number or distribution of leukocytes, number of erythrocytes, hemoglobin concentration, volume of packed red cells, number of platelets, renal function (urine analysis, phenolsulfonphthalein excretion and urea clearance), hepatic function (serum bilirubin, icteric index and bromsulfalein retention), chemical constituents of the blood (glucose, non-protein nitrogen, carbon dioxide combining power, cholesterol, calcium, phosphorus, total serum protein and albumin-globulin partition), electrocardiogram, teleroentgenogram of the chest, or basal metabolic rate. The stools did not contain occult blood even during the transient diarrhea that followed large doses of DFP and neostigmine.

Two of the patients who had received DFP daily for several weeks died 8 and 64 days after the last dose of the drug in an exacerbation of their myasthenia gravis which developed during acute infections of the respiratory tract. An autopsy was performed in each instance which disclosed purulent bronchitis and extensive areas of lobular pneumonia. There was hyperplasia of lymphatic tissue throughout the body in both cases, a lesion found not infrequently in myasthenia gravis. No other lesions were discovered on either gross or microscopic study.

## DISCUSSION

*1. The Regenerative and other Properties of Plasma Cholinesterase*

Plasma ChE activity (which is identical with serum ChE activity) has been attributed to a mucoprotein which can be separated from the other plasma proteins and crystallised (5). Cohn and his coworkers (9) found that this enzyme was associated with their plasma protein fraction IV-6, 95 per cent of which consisted of alpha<sub>2</sub> globulin of molecular weight 300,000 and 5 per cent of albumin of molecular weight 69,000 (33). The results of the study of the movement of plasma ChE into serous exudates and urine has provided some indirect information regarding certain physical properties of this enzyme protein. The diffusions of plasma ChE, serum albumin, and serum globulin into serous exudates were roughly parallel, with the diffusion of ChE being in general slightly less than that of albumin and globulin. A similar relation for the diffusions of albumin, globulin and fibrinogen into serous exudates has been described (26). In contrast to their roughly parallel diffusions into serous exudates, the movements of plasma ChE, albumin, and globulin into the urine were markedly different, with albumin being lost into the urine in very large amounts, globulin to a moderate degree, and plasma ChE in only very small amounts. Again a similar relation for the appearance of albumin, globulin, and fibrinogen in nephrotic urine has been described (25). These observations indicate that of the several membrane barriers that were studied (peritoneal, pleural, pericardial, synovial, and renal) the nephrotic kidney was the only one that showed markedly different permeability to plasma cholinesterase, globulin, and albumin. In speaking of a renal membrane barrier it is understood that the concentration of a protein in urine is the resultant of at least two functions operating in opposite directions, glomerular filtration and tubular reabsorption. Diffusion across the renal barrier as calculated here, therefore, must represent the algebraic sum of the protein filtered through the glomerulus and that reabsorbed by the tubule. Since differences in the molecular weight of proteins are a factor influencing their diffusion across membranes (in addition to other factors such as equatorial diameter, charge, etc. (8)) the data suggest that the molecular weight of plasma ChE may be higher than that of the serum albumins (69,000)

and of most of the serum globulins (169,000) and may perhaps approach that of fibrinogen (400,000).

The ChE activity of the plasma has been studied in a variety of diseases, and has been reported to be reduced in liver disease (3, 30, 13), anemia (3, 13), acute infectious diseases (16), chronic debilitating diseases (31), cardiac failure, and renal disease (13). McArdle (30) proposed that the serum ChE activity be used as an index of liver function. Faber (13) observed that reduction of serum ChE activity was frequently accompanied by a parallel reduction in serum albumin concentration, and suggested that serum ChE and serum albumin may have a common site of origin, presumably the liver. These suggestions have been supported by the report of Brauer and Root (6) that the production of liver damage in rats by the administration of carbon tetrachloride resulted in a decrease in the plasma ChE activity, and have been refuted by Steensholdt and Venndt (38), who reported an increase in serum ChE activity following the production of liver damage in dogs by chloroform. Other experiments have indicated that this difference may be due to species differences in the cholinesterases of the liver and plasma (35), which make the results of ChE studies in some animals inapplicable to man. The observations reported in this paper add support to the hypothesis which emphasises the importance of the role played by the liver in the elaboration of plasma ChE. It will be noted that in disturbances of protein metabolism due to liver damage the basal level and regenerative capacity of plasma ChE were impaired in direct proportion to the hepatic insufficiency, while in disturbances of protein metabolism associated with renal disease the activity and regenerative capacity of plasma ChE were not impaired.

The deductions drawn above from the data on the restoration of plasma and red blood cell ChE activities following their depression by DFP, rest upon two assumptions. The first is that DFP destroys ChE irreversibly. Evidence for this has been furnished by the *in vitro* studies of Mazur and Bodansky (29). The second assumption is that DFP destroys the ChE within a brief period of time and that the drug itself is inactivated quickly in the body. Again Mazur (28) has provided evidence that there is available in the body tissues an enzyme, fluorophosphatase, which inactivates DFP rapidly. In view of this evidence it may be assumed reasonably that following the cessation of the administration of DFP no ChE will be released as a result of the

reversible activation of inactivated enzyme, and that there will be no destruction of regenerated ChE by DFP which remains active.

However, Mazur (28) has shown that the liver contains a higher concentration of fluorophosphatase than do other tissues, and it might be presumed that a relative insufficiency of this enzyme in patients with hepatic damage may account for the reduction in regenerative capacity observed in these patients. Inadequate levels of fluorophosphatase may perhaps explain the more striking reduction in plasma ChE regeneration observed in these patients during the first 24 hours after the administration of DFP was stopped (Figure 4), but it is an unlikely explanation for the over-all reduction in plasma ChE regeneration, since the regeneration of red blood cell esterase was not impaired. If the effect of DFP were prolonged by fluorophosphatase deficiency then one would anticipate a persistent depression in the red blood cell ChE activity as well. Furthermore, it is possible that the more marked reduction in plasma ChE regeneration observed in these patients during the first day after the cessation of DFP may be related not to fluorophosphatase deficiency, but to a diminished hepatic store of ChE, which is reported to occur in a damaged liver (6).

Outspoken liver disease was not the only cause of depression of plasma ChE activity and regeneration, since less marked, but significant, depression was also detected in anemias, and in acute febrile and chronic debilitating diseases. In these conditions some impairment of liver function was frequently detected, and is known to be common (30), but the degree of reduction of plasma ChE activity and regeneration was greater than the degree of liver impairment that was detectable by the ordinary tests. While it is possible that plasma ChE synthesis, presumably in the liver, may be more sensitive to inhibition by anemias, and by acute febrile and chronic debilitating diseases than are the usual tests of liver function, the possibility also exists that there may be other limiting factors than liver function which control the activity and synthesis of this enzyme protein. There was no evidence that free or dialysable inhibitors of ChE activity were an important factor in any of these conditions.

## *2. The Relation of Symptoms to the Cholinesterase Activity of Plasma and Red Blood Cells*

It is very probable that the appearance and severity of cholinergic symptoms following the administration of DFP are related to the

depression of tissue cholinesterases in the effector organs themselves, and are unrelated to the ChE activity of either the plasma or the red blood cells. It is likely that the appearance of symptoms at a given level of red blood cell ChE activity following the administration of DFP for a short period of time is a coincidence, resulting from equal sensitivity of the cholinesterases in the tissues and in the red blood cells to DFP. This equality has been established *in vitro* by Mazur and Bodansky (29) for human erythrocytes and brain, in contrast to plasma ChE, which is much more sensitive to the action of DFP.

The lack of correlation between symptoms and red blood cell ChE activity following the administration of DFP in small doses over a longer period of time, or following the cessation of DFP, may be explained by two possible factors. If the regeneration of tissue cholinesterases following their depression by DFP were more rapid than that of red blood cell ChE (which is only 1.2 per cent per day) the tissue esterases might remain at a level compatible with normal function despite the progressive depression of the red blood cell ChE by small daily doses of DFP. Likewise, the tissue esterase activity might be restored appreciably within 48 hours after the cessation of DFP, thereby accounting for the disappearance of symptoms during this time, in which there is negligible regeneration of red blood cell esterase. In addition, there is the possibility that certain tissues may, through adaptation, function relatively normally at a level of tissue ChE considerably below the normal or optimal range.

The suggestion that tissue cholinesterases may be regenerated more rapidly than red blood cell ChE following their depression by DFP in human subjects is at variance with the observations of Mazur and Bodansky (29) and Koelle and Gilman (21) that in the rabbit and rat the red blood cell ChE is regenerated more rapidly. This difference between man and the rabbit or rat may perhaps be attributed to the more rapid replacement rate of red blood cells in the lower animals ( $8\frac{1}{2}$  days (12, 17) in contrast to at least 80 days in man), resulting in the more rapid regeneration rate of red blood cell ChE.

### *3. The Toxicity of DFP*

All of the symptoms and objective findings which were observed in patients who received DFP could be ascribed to the known effect of

this drug in inhibiting the action of ChE enzymes throughout the body, particularly in the autonomic and central nervous systems, and at the neuromuscular junction. There were no other toxic effects observed. However, great caution must be exercised in the use of this potent and potentially dangerous agent because of its cumulative action, and because of the long-lasting sensitisation<sup>2</sup> that follows its administration. Furthermore, the administration of lethal doses of DFP to animals (32, 22) has resulted in fatal circulatory and respiratory depression without demonstrable changes in the organs and tissues at autopsy.

We are greatly indebted to Dr. John A. Luetscher, Jr., for the electrophoretic protein analyses of the nephrotic urines that were studied and for other help, to Dr. Philip F. Wagley for his collaboration in the study of the three patients with pernicious anemia and in the experiments on folic acid, and to Dr. John R. Brewer of Hyinson, Westcott, and Dunning for his help in the ampouling of our solutions of DFP. We are also indebted to Miss Virginia L. Kremer and Miss Sylvia R. Beck for their technical assistance.

#### SUMMARY

1. The administration of DFP to human subjects caused an immediate marked depression of plasma cholinesterase activity and a much slower depression of red blood cell cholinesterase activity.

2. The cholinesterase activity of the plasma, and its regeneration following depression by DFP, were reduced in patients with liver disease in proportion to the degree of hepatic insufficiency.

3. Plasma cholinesterase activity and regeneration were also reduced in severe anemia, and in acute febrile and chronic debilitating diseases. Plasma cholinesterase activity was normal or elevated in nephrosis, and at the lower limits of normal in advanced chronic nephritis.

4. The diffusions of plasma cholinesterase, serum albumin, and serum globulin into serous exudates were roughly parallel. In contrast, their movements through the nephrotic kidney were markedly different, with albumin appearing in the urine in very large amounts,

<sup>2</sup> "Sensitisation", in this series of papers, connotes simply an apparent lowering of threshold and does not imply the existence of an allergic phenomenon.



globulin in moderate amounts, and cholinesterase in much smaller quantities.

5. The regeneration of red blood cell cholinesterase following its depression by DFP proceeded at a rate determined by the replacement rate of the red blood cells themselves (1.2 per cent daily). This rate, which was proportional to the number of circulating reticulocytes, became extremely high in patients with pernicious anemia during the early phases of specific therapy.

6. The administration of DFP caused symptoms which duplicated the muscarinic and nicotinic effects of cholinergic drugs in general, and, in addition, symptoms referable to the central nervous system. The administration of atropine inhibited the muscarine-like and central nervous system symptoms, but had no effect on the nicotine-like symptoms.

7. The symptoms caused by DFP are explicable in terms of the *inhibition of the cholinesterase enzymes of the tissues themselves, and are not related to the cholinesterase activity of the plasma or red blood cells.*

## REFERENCES

1. ADKINS, H., AND WILDE, A. L.: 1943, quoted by (19).
2. AMMON, R.: The enzymatic splitting of acetylcholine. *Pflüg. Arch. ges. Physiol.*, 1930, 233: 486.
3. ANTROPOL, W., TUCHMAN, L., AND SCHIFRIN, A.: Choline-esterase activity of human sera, with special reference to hyperthyroidism. *Proc. Soc. exp. Biol.*, N. Y., 1937, 36: 46.
4. ASHBY, W.: Determination of length of life of transfused blood corpuscles in man. *J. exp. Med.*, 1919, 29: 267.
5. BADER, R., SCHUTZ, F., AND STACEY, M.: A crystalline serum mucoprotein with high cholinesterase activity. *Nature*, 1944, 154: 183.
6. BRAUER, R. W., AND ROOT, M. A.: The effect of carbon tetrachloride induced liver injury upon the acetylcholine hydrolyzing activity of blood plasma of the rat. *J. Pharmacol.*, 1946, 88: 109.
7. CALLENDER, S. T., POWELL, E. O., AND WITTS, L. J.: The life span of the red cell in man. *J. Path. Bact.*, 1945, 57: 129.
8. COHN, E. J.: Blood, blood derivatives, and blood substitutes. *Proc. Amer. philosoph. Assoc.*, 1944, 88: 159.
9. COHN, E. J., STRONG, L. E., HUGHES, W. L., JR., MULFORD, D. J., ASHWORTH, J. N., MELIN, M., AND TAYLOR, H. L.: Preparation and properties of serum and plasma proteins. IV. *J. Amer. Chem. Soc.*, 1946, 68: 459.

10. COMROE, J. H., JR., TODD, J., AND KOELLE, G. B.: The pharmacology of DFP in man. *J. Pharmacol.*, 1946, 87: 281.
11. DAVIS, J. E.: On the mechanism of action of folic acid and liver extract in the treatment of anemia. *Science*, 1946, 104: 37.
12. EATON, P., AND DAMREN, F. L.: Method for determining life duration of the erythrocyte. *South. Med. J.*, 1930, 23: 311, 395.
13. FABER, M.: Serum cholinesterase in disease and relationship to serum albumin. *Acta med. Scand.*, 1943, 114: 59, 72.
14. GROB, D., LILIENTHAL, J. L., JR., AND HARVEY, A. M.: The administration of DFP to man, II. *Bull. Johns Hopkins Hosp.*, 1947, 81: 245.
15. GROB, D., HARVEY, A. M., LANGWORTHY, O. R., AND LILIENTHAL, J. L., JR.: The administration of DFP to man, III. *Bull. Johns Hopkins Hosp.*, 1947, 81: 257.
16. HALL, G. E., AND LUCAS, C. C.: Choline-esterase activity of normal and pathological human sera. *J. Pharmacol.*, 1937, 59: 34.
17. HARNE, O. G., LUTZ, J. F., AND DAVIS, C. L.: Induced reticulocytosis in rat and its relation to life duration of red blood cell. *J. Lab. Clin. Med.*, 1940, 25: 333.
18. HARVEY, A. M., LILIENTHAL, J. L., JR., GROB, D., JONES, B. F., AND TALBOT, S. A.: The administration of DFP to man, IV. *Bull. Johns Hopkins Hosp.*, 1947, 81: 267.
19. HORTON, R. G., KOELLE, G. B., McNAMARA, B. P., AND PRATT, H. H.: The acute toxicity of DFP. *J. Pharmacol.*, 1946, 87: 414.
20. KERR, W. J., HURWITZ, S. N., AND WHIPPLE, G. H.: Regeneration of blood serum proteins. *Amer. J. Physiol.*, 1918, 47: 356, 370, 379.
21. KOELLE, G. B., AND GILMAN, A.: The relationship between cholinesterase inhibition and the pharmacological action of DFP. *J. Pharmacol.*, 1946, 87: 421.
22. KOELLE, G. B., AND GILMAN, A.: The chronic toxicity of DFP in dogs, monkeys and rats. *J. Pharmacol.*, 1946, 87: 435.
23. LANGE, W., AND VON KRUEGER, G.: On esters of monofluorophosphoric acid. *Ber. dtsch. chem. Ges.*, 1932, 65: Part II: 1598.
24. LUCAS, C. C., HALL, G. E., AND ETTINGER, C. H.: Individual and species variations in the choline esterase and other esterases of blood serum. *J. Pharmacol.*, 1935, 54: 151.
25. LUETSCHER, J. A., JR.: Electrophoretic analysis of plasma and urinary proteins. *J. clin. Invest.*, 1940, 19: 313.
26. LUETSCHER, J. A., JR.: Electrophoretic analysis of the proteins of plasma and serous effusions. *J. clin. Invest.*, 1941, 20: 99.
27. MACKWORTH, J. P.: 1942, quoted by (29).
28. MAZUR, A.: An enzyme in animal tissues capable of hydrolyzing the phosphorus-fluorine bond of alkyl fluorophosphates. *J. biol. Chem.*, 1946, 164: 271.
29. MAZUR, A., AND BODANSKY, O.: The mechanism of in vitro and in vivo inhibition of cholinesterase activity by DFP. *J. biol. Chem.*, 1946, 163: 261.

30. MCARDLE, B.: Serum choline esterase in jaundice and diseases of liver. *Quart. J. Med.*, 1940, **9**: 107.
31. MILHORAT, A. T.: The choline-esterase activity of the blood serum in disease. *J. clin. Invest.*, 1938, **17**: 649.
32. MODEL, W., KROP, S., HITCHCOCK, P., AND RIKER, W. K., JR.: General systemic actions of DFP in cats. *J. Pharmacol.*, 1946, **87**: 400.
33. ONCLEY, L., SCATCHARD, G., AND BROWN, A.: Physical-chemical characteristics of certain of the proteins of normal human plasma. *J. physical colloid Chem.*, 1947, **51**: 184.
34. SABINE, J. C.: Choline esterase of blood cells and plasma in blood dyscrasias, with special reference to pernicious anemia. *J. clin. Invest.*, 1940, **19**: 833.
35. SAWYER, C. H.: Hydrolysis of choline esters by liver. *Science*, 1945, **101**: 385.
36. SHEMIN, D., AND RITTENBERG, R.: The life span of the human red blood cell (study employing isotopes). *J. biol. Chem.*, 1946, **166**: 627.
37. STANBURY, J. B., WARWEG, E., AND AMBERSON, W. R.: Total plasmaphoresis. *Amer. J. Physiol.*, 1936, **117**: 230.
38. STEENSHOLDT, G., AND VENNDT, H.: On serum cholinesterase activity in experimental liver injury. *Acta physiol. Scand.*, 1945, **10**: 23.
39. VAHLQUIST, B.: On esterase activity of human blood plasma. *Skand. Arch. Physiol.*, 1935, **72**: 133.
40. WEARN, J. T., WARREN, S., AND AMES, O.: Length of life of transfused erythrocytes. *Arch. intern. Med.*, 1922, **29**: 527.
41. WIENER, A. S.: Longevity of the erythrocyte. *J. Amer. med. Ass.*, 1934, **102**: 1779.

# THE ADMINISTRATION OF DI-ISOPROPYL FLUOROPHOSPHATE (DFP) TO MAN

## II. EFFECT ON INTESTINAL MOTILITY AND USE IN THE TREATMENT OF ABDOMINAL DISTENTION<sup>1</sup>

D. GROB, J. L. LILIENTHAL, JR., AND A. M. HARVEY

*From the Physiological Division, Department of Medicine, Johns Hopkins University and Hospital, Baltimore, Maryland*

Di-isopropyl fluorophosphate (DFP) is a potent anticholinesterase agent which permanently inactivates cholinesterase (ChE) with which it comes in contact (6). The general systemic effects of DFP, and its effect on plasma and red blood cell ChE activity have been presented in a previous report (2). Following the daily intramuscular administration of DFP to 60 normal subjects gastrointestinal symptoms usually occurred, including, in order of frequency, anorexia, nausea, abdominal cramps, vomiting, diarrhea, and symptoms of cardiospasm. It was observed that these symptoms were increased by the administration of neostigmine or pitressin in doses that had been ineffective before the administration of DFP, and were suppressed by atropine. The administration of neostigmine shortly before DFP was observed to diminish somewhat the symptoms produced by the latter.

### THE EFFECT OF DFP ON INTESTINAL ACTIVITY

Intestinal activity was recorded in 26 experiments on three subjects by means of a water manometer-air tambour recorder which was connected to a water-filled Miller-Abbott balloon lying in the intestine. Such a recorder does not measure absolute intraluminal pressure with accuracy (1) but does record changes in intestinal motility and tone.

The intramuscular injection of 1 to 3 mg. of DFP in 0.1 per cent solution in peanut oil caused a marked increase in the motility of the small and large intestine, with rhythmic contractions of greatly increased amplitude beginning about one hour after injection of the drug and occurring at the rate of one every two or three minutes over a

<sup>1</sup> Work performed under a contract between the Medical Division, Chemical Corps, U. S. Army, and the Johns Hopkins University.

period of two to five hours (Figure 1). The frequency of the contractions was greatest initially and decreased with time. Occasionally, relaxation following a muscular contraction was slow, with the contraction persisting for several minutes, but, in general, the resting tone of the intestine was not changed during the period of augmented activity. During this period of increased intestinal activity numerous borborygmi were audible over the abdomen, and with the larger dose of 3 mg. nausea and abdominal cramps sometimes appeared.

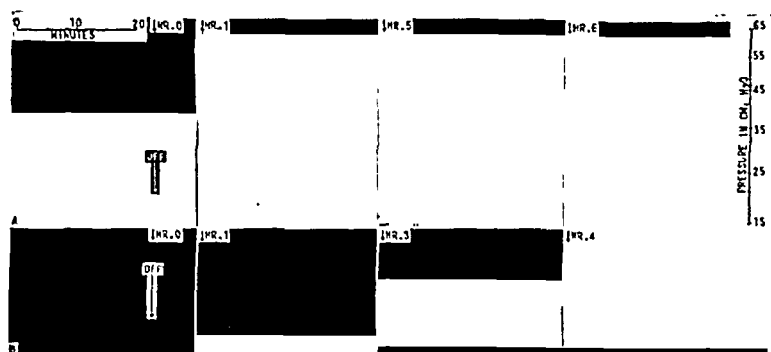


FIG. 1. THE EFFECT OF DFP ON THE MOTILITY OF THE ILEUM AND COLON

A. DFP, 1 mg. in oil, administered intramuscularly to subject T. D. (ileostomy). Rhythmic contractions of the ileum began one hour later and persisted for five hours. B. DFP, 2 mg. in oil, administered intramuscularly to subject G. J. (co-stomy). Rhythmic contractions of the colon began one hour later and persisted for three hours.

#### INHIBITION OF THE DFP EFFECT

The increased intestinal motility due to DFP could be inhibited temporarily by the administration of atropine, morphine, or demerol (isonipecaine, meperidine) (Figure 2). Atropine and demerol, which decreased the tone and motility of the normal small intestine, had a similar effect during the activity induced by DFP. Morphine, which increased the tone and decreased to some extent the motility of the normal small intestine, likewise had a similar effect after the administration of DFP. The diminution of motility was greater following atropine or demerol than after morphine.

#### POTENTIATION OF THE ACTION OF OTHER DRUGS BY DFP

Since DFP inhibits cholinesterase irreversibly, it would be expected to exert an effect until the enzyme has regenerated to a level compatible



FIG. 2. THE INHIBITION BY ATROPINE, MORPHINE, AND DEMEROL OF THE INCREASED INTESTINAL MOTILITY DUE TO DFP (SUBJECT T. D., ILEOSTOMY)

*A and D.* The effect of atropine (1.2 mg. intravenously) and morphine (12 mg. intravenously) on the normal intestine. The effect of demerol (50 mg. intravenously) was the same as that of atropine. *B, E, and G.* The effect of atropine, morphine, and demerol (in the above doses) on the increased intestinal motility due to DFP (2 mg. administered intramuscularly one hour (*B*), 1½ hours (*D*), and two hours (*G*) previously). *C, F, and G.* The return of increased intestinal motility 80 minutes (*C*), 87 minutes (*F*), and 30 minutes (*G*) later.

with normal function. Twenty-four hours after the last dose of DFP the intestinal motility was still slightly increased, as manifested by the increased frequency and amplitude of the small rhythmic waves. (Figure 3B). In addition, the intestine was found to be sensitised to

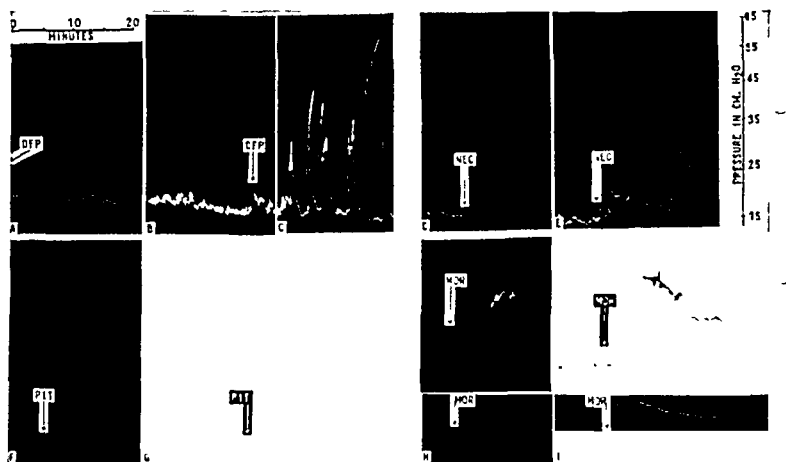


FIG. 3. THE POTENTIATION BY DFP OF THE ACTION OF DFP, NEOSTIGMINE, PITRESSIN, AND MORPHINE ON THE ILEUM. (SUBJECT T. D., ILEOSTOMY)

A, DFP, 0.1 mg. I.M.; D, neostigmine, 0.05 mg. I.V.; F, pitressin, 1 unit I.V.; H (lower), morphine, 3 mg. I.V.: Subeffective doses, administered before the patient received DFP. B, DFP, 0.1 mg. I.M.; E, neostigmine, 0.05 mg. I.V.; G, pitressin, 1 unit I.V.; I (lower), morphine, 3 mg. I.V.: The same doses administered 24 to 40 hours after the last dose of DFP (2 mg. q.d. for 2 days), at which time ChE activity was approximately 5 per cent (plasma) and 50 per cent (red blood cells) of control. DFP, neostigmine and pitressin now cause increased intestinal motility and cramps, while the morphine now causes increased intestinal tone. H (upper), morphine 12 mg. I.V. administered before the patient received DFP. I (upper), morphine 12 mg. I.V. administered 48 hours after the last dose of DFP.

the action of DFP, neostigmine, pitressin, morphine and inhaled tobacco smoke (Figures 3 and 4). The potentiation of the action of DFP, neostigmine and pitressin was most striking. Doses of these drugs which had produced no effect before the administration of DFP now caused intestinal activity sufficient to produce nausea and abdominal cramps after sensitisation of the intestine by DFP. The potentiation

of the action of neostigmine was also demonstrable when injected during augmented intestinal activity due to DFP. A previously ineffective dose of neostigmine resulted in increased intestinal activity with the development of abdominal cramps (Figure 5).

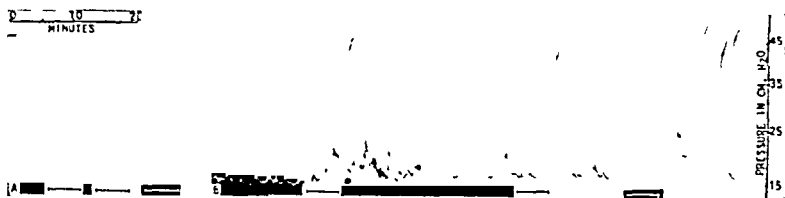


FIG. 4. THE POTENTIATION BY DFP OF THE EFFECT OF SMOKING ON THE ILEUM (SUBJECT T. D., ILEOSTOMY)

- A. Slight effect of smoking three cigarettes before the subject received DFP.  
 B. Increased intestinal motility and cramps following the smoking of three cigarettes 24 hours after the last dose of DFP (2 mg. q.d. for two days).

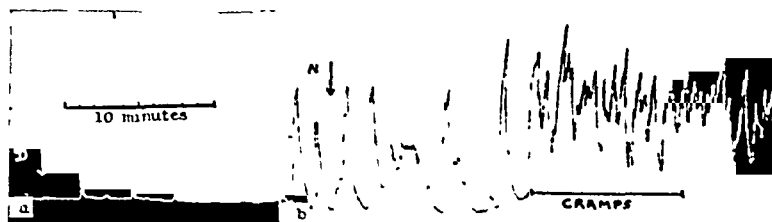


FIG. 5. THE POTENTIATION OF THE ACTION OF NEOSTIGMINE ON THE JEJUNUM BY DFP DURING THE TIME OF INCREASED MOTILITY DUE TO DFP (SUBJECT W. W.)

- A. DFP, 2 mg. in oil, intramuscularly. B. Rhythmic contractions, which began one hour after (a), recorded two hours after administration of DFP. A previously ineffective dose of neostigmine (1 mg. intramuscularly) now causes increased motility and cramps.

The duration of this increased sensitivity of the intestine to stimulating drugs was investigated in 15 subjects by the daily administration of graded doses of neostigmine and pitressin before, during, and after the period of injection of DFP, with determination of the intramuscular dose required to produce abdominal cramps. Doses of neostigmine and of pitressin which produced no symptoms previously caused abdominal cramps, nausea, and frequently vomiting and diarrhea when



given during, or for a period of one to three weeks after, the administration of DFP (Figure 6).

#### THE TREATMENT OF ABDOMINAL DISTENSION WITH DFP

The prolonged effect of DFP on intestinal motility and its potentiation of the effects of neostigmine and pitressin suggested a therapeutic trial of this drug in the treatment of abdominal distension. Sixty-four

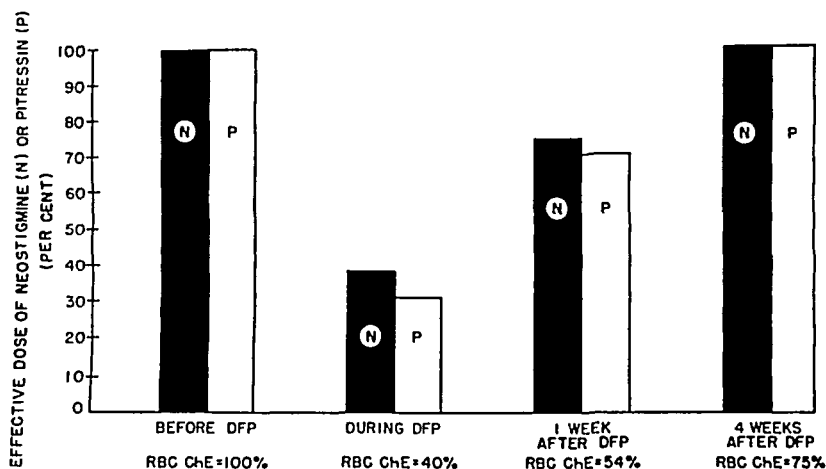


FIG. 6. The increased sensitivity of the gastrointestinal tract to intramuscular neostigmine and pitressin during and after the daily intramuscular administration of DFP to 11 subjects (eight normal and three myasthenic). The effective dose is that required to produce abdominal cramps. Each block represents the average of the 11 subjects. The results were similar in normal and myasthenic subjects except that the latter were less sensitive to neostigmine than the former.

patients with moderate to severe paralytic ileus were treated with intramuscular DFP, supplemented in 46 of the more severe cases by the hypodermic injection of neostigmine. Forty-six of these patients had distension following major abdominal operations, eight had peritonitis (tuberculous (2), post-operative (2), gonococcal (1), and due to perforating wound of the abdomen (3)), five had severe pneumonia, and five had lesions of the spinal cord (due to transverse myelitis, syringomyelia, epidural abscess, ruptured intervertebral disc, and vertebral

fracture). The distension in these patients had not responded to enemas and the use of a rectal tube. The 46 more severe cases (28 of the post-operative cases and all the remainder) had also been given 0.5 to 1.0 mg. neostigmine (supplemented in 25 cases by 10 to 20 units of pitressin) every two hours without adequate effect.

In the less severe cases relief was obtained from the intramuscular administration of DFP alone. One to two hours after the initial dose of 2 mg. of DFP there was evidence of increased intestinal motility, manifested by borborygmi, the passage of flatus, decreased nausea, and some relief of the distension. This persisted for from two to eight hours. The subsequent administration of DFP (at 8 to 24 hour intervals) resulted in evidence of increasing intestinal motility, with the frequent appearance of mild cramps, spontaneous defecation, and subsidence of the distension. The effect of the DFP (in peanut oil) was much more prolonged than that of neostigmine or pitressin (in aqueous solution), and was rarely, in the doses employed, accompanied by troublesome abdominal cramps.

In the more severe cases, whose distension was not fully relieved by DFP alone, good results were obtained by the additional use of neostigmine in doses which had previously been ineffectual. The administration of 0.5 to 1 mg. of this drug was followed by the passage of flatus and frequently by a bowel movement, with marked lessening of the distension. The administration of pitressin (10 to 20 units) produced a similar effect, and the combined use of the two drugs following sensitisation of the intestine by DFP produced the best results.

The optimal dosage of DFP, and the best combination with other drugs, has not yet been fully determined. The following schedule has produced satisfactory results within 4 to 24 hours after the onset of therapy:

An initial dose of 2 mg. of DFP was administered, followed in three and seven hours by neostigmine (0.5 to 1 mg. hypodermically) and pitressin (10 to 20 units hypodermically). One mg. of DFP was then administered at 12 hour intervals for two or three doses, with neostigmine and pitressin three and sometimes seven hours after each injection. One mg. of DFP was injected on subsequent days if necessary to prevent or treat the recurrence of distension, and neostigmine and pitressin were added as needed.

In order to obtain the maximum effect of DFP in the treatment of abdominal distension, it proved desirable to withhold drugs which suppress the increased intestinal motility that follows the administration of DFP. Thus, atropine and demerol were not given, unless necessary to control symptoms of DFP overdose. The administration of morphine to control pain was timed when possible so as not to coincide with the period of maximum action of DFP. Finally, in view of the observation that the administration of neostigmine shortly before DFP diminished the anticipated symptoms of the latter, DFP was not administered until three or more hours after the last dose of neostigmine. On the other hand, neostigmine was given at any time after the action of DFP had begun, and usually was a necessary adjunct in the treatment of moderate or severe distension.

The potent actions of DFP made necessary careful regulation of dosage and close watch for the development of central nervous system symptoms. Of the 64 patients treated with DFP for abdominal distension in the doses described above, the only untoward symptoms that were encountered were excessive dreaming in eight patients, nightmares in three, vertigo in one, paresthesias in one, and increased delirium in one patient severely ill with pneumonia. These symptoms all disappeared within 48 hours after the cessation of DFP.

In order to avoid the unpleasant and potentially dangerous side effects of DFP the amount of DFP administered on the first day should not exceed 3 or 4 mg., and on subsequent days 1 or 2 mg. In most instances the drug should be discontinued after one week of daily administration, or a total dose of 10 mg. Should further administration be desired the daily dose should be reduced below 1 mg., and the patient followed with extreme care, preferably with the help of serial determinations of the red blood cell ChE activity. Since the effective intramuscular dose of DFP is related to body weight, the dosage should be less in small or emaciated patients.

It must be emphasized that under no circumstances should this drug be given to a patient until the possibility of an organic obstruction of the gastrointestinal tract has been excluded. If vomiting or abdominal pain occurs due to DFP this can be readily inhibited by the administration of atropine.

## DISCUSSION

*Mode of Action of DFP*

The increased intestinal motility that follows the administration of DFP is believed to be due to the irreversible inhibition by DFP of part of the ChE localised in the intestine. Measurement of the local concentration of ChE was not possible, but the time relation of the in-

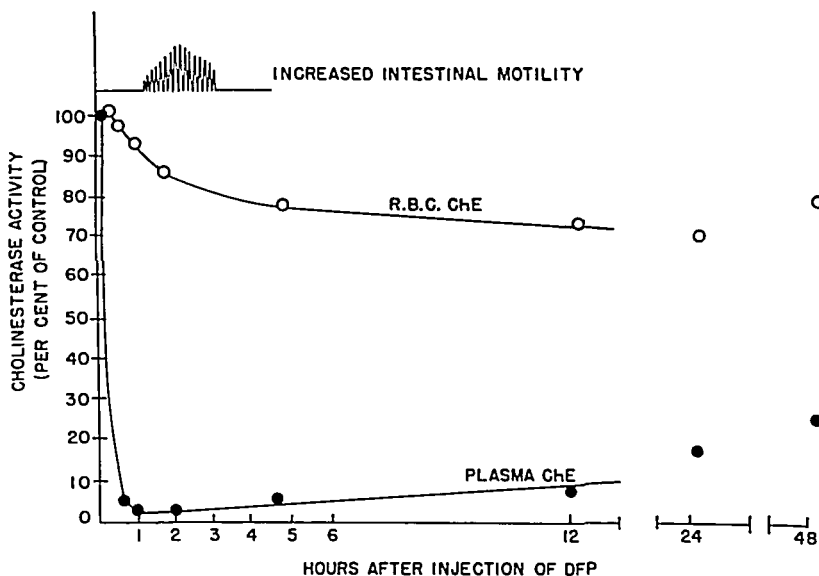


FIG. 7. The effect of a single intramuscular injection of DFP in oil (2 mg.) on cholinesterase activity (plasma and red blood cell) and intestinal motility. (Subject W. W.). The upper line represents schematically the appearance of intestinal hyperactivity.

creased intestinal motility to the concomitant depression of plasma and red blood cell ChE was determined (Figure 7). Evidence obtained from the study of experimental animals (5), and the correlation in human subjects between red blood cell ChE activity and the onset of symptoms following a brief period of administration of DFP (2), suggest that following a single dose of DFP the reduction of tissue ChE concentration probably is indicated better by the fall in red blood cell rather than plasma activity.

The subsidence of intestinal overactivity within three to six hours after the administration of DFP cannot be due to local regeneration of ChE to normal levels, for this has been shown to require many days (6). This disappearance of spontaneous overactivity may perhaps represent a process of local adaptation with a rapid initial regeneration of tissue ChE to a level where acetylcholine is hydrolysed at relatively normal rates. The slow disappearance of hypersensitivity of the gastrointestinal tract during the one to three weeks after DFP administration probably does reflect the local regeneration of ChE to normal levels, and may be compared with a similar process occurring in relation to neuromuscular function and electrical activity of the brain after cessation of DFP (3, 4).

The observation that the administration of neostigmine shortly before DFP diminished the anticipated symptoms of the latter parallels a similar phenomenon observed in the study of neuromuscular function (4) and illustrates a difference in the mode of action of these drugs. It is believed that neostigmine, in contrast to DFP, combines reversibly with ChE, and that the ChE so bound is not susceptible of destruction by DFP. On the other hand when neostigmine is administered after DFP has exerted its effect on the intestinal ChE (Figures 3E and 5) further increase in intestinal activity is produced, presumably because of the inhibition by neostigmine of the remaining local ChE that has escaped destruction by DFP.

The observation that DFP potentiates the action of pitressin and of morphine on the intestinal tract is of considerable physiological interest, since the latter drugs are said to exert their effect by direct action on smooth muscle itself in contrast to the anticholinesterase compounds, DFP and neostigmine, which are thought to act primarily at the nerve-ending.

#### *Necessary Precautions in the Use of DFP*

It must be emphasised that DFP exerts a cumulative effect which is very slowly reversible, and that in overdose in experimental animals death follows the development of respiratory and circulatory failure, probably central in origin (7). In man it has been observed invariably that repeated doses of DFP exert an increasing effect, presumably because of the progressive irreversible inhibition of ChE. Similarly,

since ChE is slowly regenerated after the cessation of DFP the sensitisation produced by this drug is long lasting, so that should further administration of DFP be desirable within three or four weeks after the last injection of the drug, the dose must be reduced. Following repeated administration of DFP the red blood cell ChE activity gives only a very approximate indication of the state of the tissue ChE as it is apparently more slowly regenerated. The plasma ChE activity probably bears no relation at all to symptoms or to sensitisation. Therefore, these determinations are of limited practical use in regulating dosage (2).

DFP has not caused peripheral vasodilatation or fall in blood pressure, even in the relatively large dose of 3 mg. a day for three days. It has caused bradycardia (with premature contractions) in only one case. Clinical observations to date have not indicated that the supplementary use of neostigmine in moderate doses results in a dangerous decrease of blood pressure or in marked bradycardia. However, animal experiments have demonstrated that hypotension following acetylcholine is considerably potentiated by the previous administration of DFP (7). This suggests that, unless further investigations indicate otherwise, acetylcholine and other direct cholinergic agents should be withheld from patients receiving DFP.

The occurrence of increased delirium following the administration of DFP to one severely ill patient may or may not be attributable to the drug, but indicates the advisability of cautious administration of DFP to severely ill patients. Similarly, although DFP has been administered to two patients with a history of asthma without untoward results the occurrence of bronchoconstriction following the injection of large doses of DFP in experimental animals (6) indicates that caution should be exercised when the drug is used in patients with a history of allergic disease.

#### SUMMARY

(1) The administration of DFP, a potent anticholinesterase drug, to human subjects caused a marked increase in the motility of the small and large intestine, which lasted, after a single injection, for two to five hours.

(2) The increased motility due to DFP was inhibited temporarily

by atropine, morphine, and demerol. It was increased by neostigmine.

(3) The administration of DFP sensitised the intestine to the action of DFP, neostigmine, pitressin, morphine, and inhaled tobacco smoke. This sensitisation lasted for one to three weeks and is believed to be due to the irreversible inactivation by DFP of intestinal cholinesterase, which then is regenerated slowly.

(4) Sixty-four patients with abdominal distension were treated successfully with DFP, supplemented in most cases by neostigmine. The best results were obtained by administering neostigmine and pitressin after sensitisation of the intestine by DFP.

(5) The central nervous system symptoms that may be caused by DFP were not prominent with the doses of the drug that were employed. However, because the repeated administration of DFP results in a cumulative irreversible inhibition of cholinesterase, and thus has potentially dangerous effects, caution should be exercised in its use.

*Acknowledgement:* We are indebted to Dr. Myron I. Buchman of the Gynecological Service for his assistance in treating many of the cases of abdominal distension, and to Dr. John H. Brewer of Hynson, Westcott, and Dunning for the ampouling of the DFP solutions that were employed.

#### REFERENCES

1. BRODY, D. A., WERLE, J. M., MESCHEN, I., AND QUIGLEY, J. P.: Intralumen pressures of the digestive tract. *Amer. J. Physiol.*, 1940, **130**: 791.
2. GROB, D., LILIENTHAL, J. L., JR., HARVEY, A. M., AND JONES, B. F.: The administration of DFP to man, I. *Bull. Johns Hopkins Hosp.*, 1947, **81**: 217.
3. GROB, D., HARVEY, A. M., LANGWORTHY, O. R., AND LILIENTHAL, J. L., JR.: The administration of DFP to man, III. *Bull. Johns Hopkins Hosp.*, 1947, **81**: 257.
4. HARVEY, A. M., LILIENTHAL, J. L., JR., GROB, D., JONES, B. F., AND TALBOT, S. A.: The administration of DFP to man, IV. *Bull. Johns Hopkins Hosp.*, 1947, **81**: 267.
5. KOELLE, G. B. AND GILMAN, A.: The relationship between cholinesterase inhibition and the pharmacological action of DFP. *J. Pharmacol.*, 1946, **87**: 421.
6. MAZUR, A., AND BODANSKY, O.: The mechanism of in vitro and in vivo inhibition of cholinesterase activity by DFP. *J. biol. Chem.*, 1946, **163**: 261.
7. MODELL, W., KROP, S., HITCHCOCK, P., AND RIKER, W. F., JR.: General systemic actions of DFP in cats. *J. Pharmacol.*, 1946, **87**: 400.

## THE ADMINISTRATION OF DI-ISOPROPYL FLUOROPHOSPHATE (DFP) TO MAN

### III. EFFECT ON THE CENTRAL NERVOUS SYSTEM WITH SPECIAL REFERENCE TO THE ELECTRICAL ACTIVITY OF THE BRAIN<sup>1</sup>

D. GROB, A. M. HARVEY, O. R. LANGWORTHY AND J. L. LILIENTHAL, JR.

*From the Physiological Division, Department of Medicine, and the Department of Psychiatry,  
Johns Hopkins University and Hospital, Baltimore, Maryland*

The general systemic effects of the potent anticholinesterase compound, di-isopropyl fluorophosphate (DFP), and its effects on plasma and red blood cell cholinesterase activity and on intestinal motility have been presented in previous reports (6, 7). The daily intramuscular administration of DFP to 60 normal subjects usually resulted in the development of symptoms referable to the central nervous system, including, in order of frequency, excessive dreaming, insomnia, jitteriness and restlessness, increased tension, emotional lability, subjective tremulousness, nightmares, headache, increased libido, giddiness, drowsiness, paresthesias, mental confusion, visual hallucinations, tremor, and pains in the legs of sciatic distribution. These symptoms were not significantly affected by the administration of neostigmine. They were diminished to some degree by the administration of atropine and by barbituric acid derivatives.

The production by DFP of symptoms which seemed to reflect increased neural activity suggested a study of the electroencephalographic changes that might follow the administration of this drug. This study was performed by means of a six channel Grass electroencephalograph employing both unipolar and bipolar leads, recordings being made before and during hyperventilation. Twenty-three subjects were observed, of whom nineteen had no demonstrable disease of the central nervous system or any neuromuscular abnormality, while four had myasthenia gravis, with normal electroencephalographic patterns.

<sup>1</sup> Work performed under a contract between the Medical Division, Chemical Corps, U. S. Army, and the Johns Hopkins University.



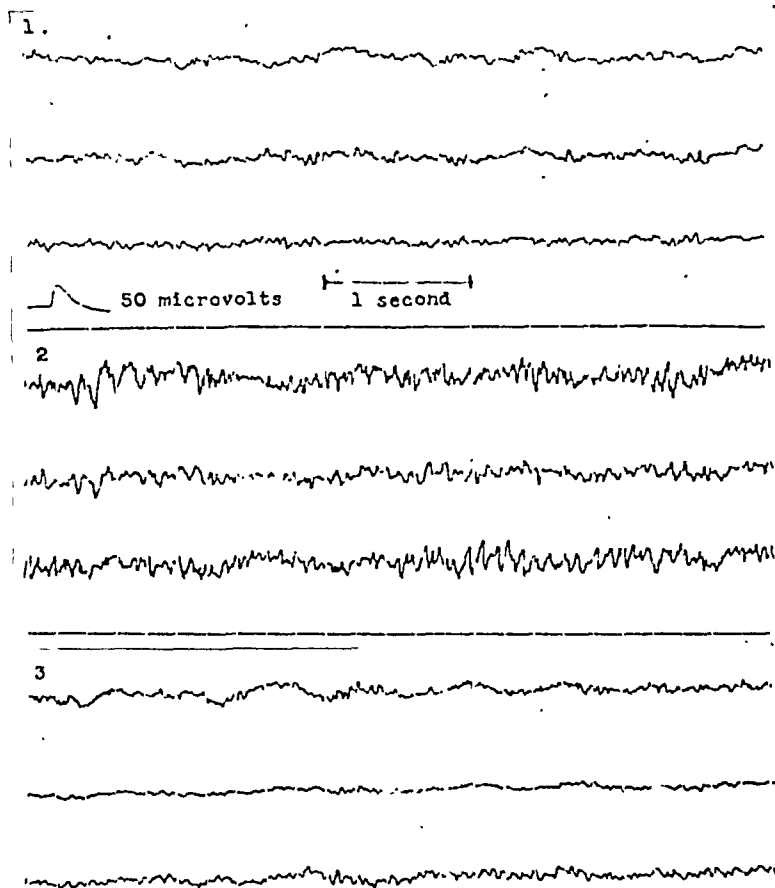


FIG. 1. Increased electrical activity of the brain following the administration of DFP to a normal subject (C. W.) and the antagonistic action of atropine. Unipolar leads: left frontal, central, and occipital leads recorded in that order. Amplification equal in all records. No hyperventilation. 1. Electroencephalogram before DFP. 2. Following the intramuscular administration of 1.5 mg. of DFP daily for three days. Cholinesterase 3 per cent (plasma) and 31 per cent (red blood cell) of original activity. 3. Five minutes after the intravenous administration of 1.2 mg. of atropine, and ten minutes after record 2.

#### THE EFFECT OF DFP ON THE ELECTROENCEPHALOGRAM

The administration of 1 to 2 mg. of DFP daily (intramuscularly) resulted in increased electrical activity of the brain in 17 of the 23 subjects (Figures 1 and 2). This was manifested by greater variations in

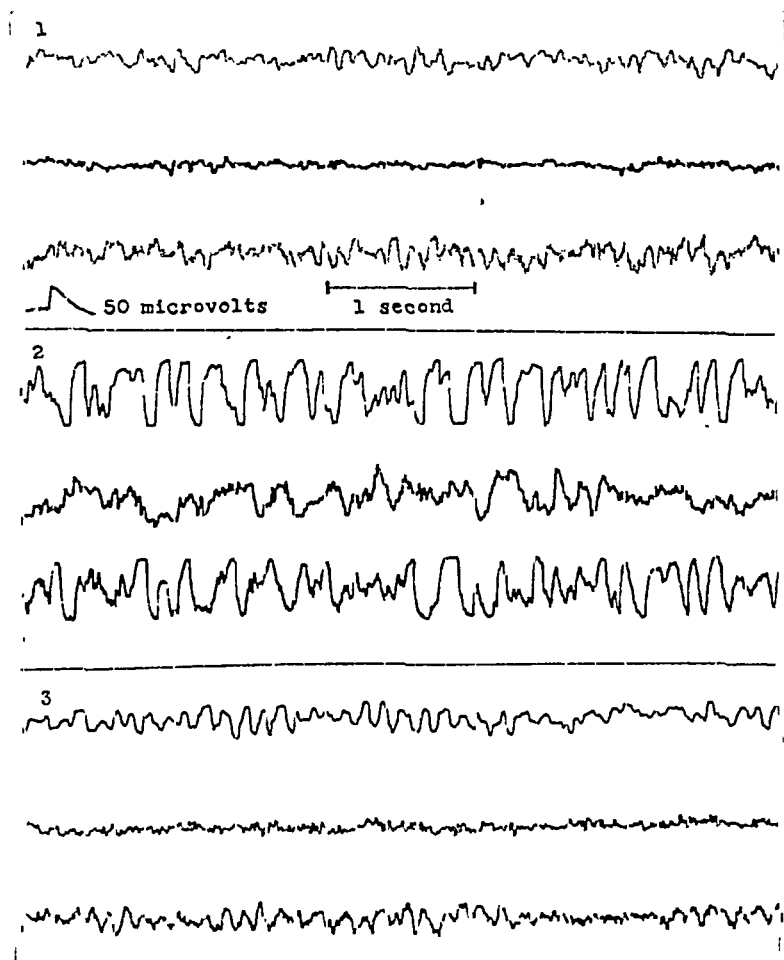


FIG. 2. Increased electrical activity of the brain following the administration of DFP to a normal subject (E. S.), and the antagonistic action of atropine. Bipolar leads: right central-frontal, frontal-frontal, and left central-frontal leads recorded in that order during the third minute of hyperventilation. Amplification equal in all records. 1. Electroencephalogram before DFP. 2. Following the intramuscular administration of 1.4 mg. of DFP daily for five days. Cholinesterase 12 per cent (plasma) and 30 per cent (red blood cell) of original activity. 3. Five minutes after the intravenous administration of 1.2 mg. of atropine, and ten minutes after record 2.

potential, by increased frequency (with increased beta rhythm), by more irregularities in rhythm, and by the intermittent appearance of

abnormal waves similar to those seen in patients with grand mal epilepsy. The latter consisted of slow waves (3 to 6 per second) of high voltage, usually most marked in the frontal leads, and increased by hyperventilation. In general, more striking electroencephalographic changes due to DFP occurred in those subjects who showed greater lability of pattern, though within the limits of normal, in their control records (Figure 2).

The electroencephalographic changes appeared after two to seven days of administration of DFP, and usually followed the onset of central nervous system symptoms. Following the cessation of DFP the symptoms due to this drug disappeared within one to four days (average two days), while the electroencephalographic changes persisted in diminishing degree for from 8 to 42 days (average 29 days).

#### CORRELATION WITH PLASMA AND RED BLOOD CELL CHOLINESTERASE

The onset or severity of central nervous system symptoms and electroencephalographic changes due to DFP bore no relation to the cholinesterase (ChE) activity of the plasma. When DFP was administered over a relatively short period of time (up to three days) the onset of symptoms and electroencephalographic changes could usually be correlated with the depression of red blood cell ChE to an average of 70 and 60 per cent of original activity, respectively. However, when DFP was administered over a longer period of time, or when administration of the drug was stopped, this correlation between central nervous system effects and red blood cell ChE activity no longer existed.

#### THE EFFECT OF ATROPINE ON CHANGES DUE TO DFP

The increased electrical activity of the brain due to DFP was inhibited in all of 17 subjects by the administration of atropine (Figures 1 and 2). The intravenous injection of 1.2 mg. of atropine to subjects who had received DFP resulted in an immediate decrease in potential and frequency (with decreased beta and increased alpha rhythm), a decrease in the irregularities of rhythm, and a decrease in the incidence of abnormal waves both before and during hyperventilation.

The daily administration of atropine, by any route, concomitant with the daily administration of DFP delayed the onset of both

central nervous system symptoms and electroencephalographic changes, the former for a few days and the latter for as long as three to four weeks, at which time the red blood cell ChE had been reduced to an average of 33 per cent of the original activity. Some of the subjects who received atropine and DFP daily for several weeks had pronounced central nervous system symptoms despite the absence of electroencephalographic changes. Withdrawal of atropine was followed within several days by the appearance of the electroencephalographic abnormalities.

#### THE EFFECT OF ATROPINE ON THE NORMAL ELECTROENCEPHALOGRAM

The administration of 1.2 mg. of atropine intravenously to 39 normal subjects resulted in some evidence of reduced electrical activity of the brain in over one-third of the subjects. In these cases there was an immediate decrease in voltage and frequency (with decreased beta and increased alpha rhythm), a decrease in the irregularities of rhythm, and a decrease in the appearance of abnormal slow waves during hyperventilation. Darrow (2) has reported previously that atropine produces changes in the normal electroencephalogram.

The administration of the same dose of atropine to 16 subjects with a history of grand mal epilepsy and with an electroencephalographic pattern characteristic of this disease resulted in some reduction of electrical activity and of abnormal waves in one-half of the subjects. These changes, which occurred immediately after the intravenous injection, were similar to those described as occurring in the normal subjects and in those who had received DFP, but were less marked than in the latter and were dramatic in only one case.

#### THE EFFECT OF NEOSTIGMINE AND CURARE ON CHANGES DUE TO DFP

The intravenous administration of 1.5 mg. of neostigmine, or 25 to 60 units of curare (Intocostin, Squibb) or d-tubocurarine (Squibb) to 26 normal subjects, and to 13 subjects who had received DFP, resulted in no change in the normal electroencephalographic pattern, or in the central nervous system symptoms or electroencephalographic abnormalities due to DFP. The doses of curare and tubocurarine that were employed were sufficient to cause subjective weakness of the facial and ocular muscles, and with the highest dose objective weakness as well.

## OTHER CENTRAL NERVOUS SYSTEM EFFECTS OF DFP

In addition to the symptoms and electroencephalographic changes which it produced, the administration of DFP resulted in other manifestations of increased central nervous system activity. These consisted of evidence of increased spinal cord activity and of spontaneous discharges within the spinal cord in two patients with upper motor neurone lesions due to late central nervous system syphilis. These patients had spastic paraplegia of the lower extremities, and no disturbance in the innervation of the upper extremities. The administration of DFP (2 mg. intramuscularly daily for three days) resulted in the familiar gastrointestinal, sudorific, and central nervous system symptoms. In addition to this, both patients developed intermittent and involuntary spontaneous clonic movements of the thigh and calf muscles which were increased greatly by passive stretching of the muscles and by voluntary movement. At the same time the weakness of the lower legs was increased and there was greater stiffness on walking. The spontaneous clonic movements, weakness, and stiffness were localized to the lower part of the body, and were only slightly inhibited by large doses of atropine. These symptoms subsided over a period of 72 hours after the last dose of DFP. They could not be reproduced by the administration of large doses of neostigmine (up to 200 mg. daily by mouth).

## DISCUSSION

*Mode of Action of DFP*

The central nervous system symptoms, electroencephalographic changes, and evidence of spontaneous discharges within the spinal cord that resulted from the administration of DFP may be interpreted to result from the irreversible inhibition by DFP of ChE in the central nervous system. Measurement of this activity was not possible in man, but animal experiments have demonstrated the inactivation of brain ChE following the administration of DFP (8; 10). The persistence of the electroencephalographic changes due to DFP for as long as three and four weeks after the last dose of the drug probably reflects the slow regeneration of ChE in the central nervous system.

When the central nervous system symptoms and electroencephalo-

graphic changes due to DFP were evident there were no alterations in the respiratory rate, pulse rate, blood pressure, or carbon dioxide combining power of the serum to which these changes might be related.

*Correlation of the Central Nervous System Effects of DFP with Plasma and Red Blood Cell Cholinesterase*

The central nervous system effects of DFP bore no relation to the ChE activity of the plasma. They could be correlated with the ChE activity of the red blood cells only during the first three days of DFP administration, suggesting that the ChE enzymes of the central nervous system and red blood cells may have similar sensitivity to DFP. However, when DFP was administered over a longer period of time, or when the administration of the drug was stopped, this correlation no longer existed, presumably because of different rates of regeneration of the ChE enzymes in the central nervous system and in the red blood cells.

*The Effect of Atropine, Curare, and Neostigmine on the Changes Due to DFP*

Feldberg (3) has pointed out that, although there are many discordant findings, atropine is in general believed to inhibit the central nervous system effects of cholinergic drugs. The observed inhibitory action of atropine on the central nervous system symptoms and electroencephalographic abnormalities due to DFP is consonant with this view.

The persistence of the electroencephalographic changes due to DFP during sufficient curarisation to cause relaxation of the frontalis muscles makes it unlikely that the changes observed in the electroencephalogram were the result of spontaneous muscle activity underlying the electrodes. In addition, these observed changes did not resemble the effects of muscle activity.

The failure of neostigmine to duplicate or increase the central nervous system effects of DFP is in direct contrast to the effect of neostigmine on the gastrointestinal tract (7). This difference may be related to the lipid solubility of neostigmine, which is much less than that of DFP. In view of the high lipid content of the central nervous

system, the different effects of neostigmine and DFP might be due in part to the different solubilities of the two compounds, which would permit DFP to penetrate the central nervous system to a greater extent than neostigmine.

### *The Effect of an Upper Motor Neurone Lesion on the Action of DFP*

The increased spinal cord activity and spontaneous discharges within the spinal cord which followed the administration of DFP to subjects with upper motor neurone lesions are compatible with the view that the central nervous system effects of DFP are mediated by increased cholinergic activity. Upper motor neurone section has been demonstrated to render the anterior horn cells (1), and the skeletal muscles supplied by such "denervated" anterior horn cells (9), more sensitive to stimulation by acetylcholine.

### *The Physiological Significance of the Central Nervous System Effects of DFP*

A variety of experiments have been performed in an attempt to demonstrate the relationship of acetylcholine to the function of the central nervous system. These have been summarised in detail by Feldberg (3). The evidence is conflicting and the experimental procedures have usually been rather complicated. In the present study central nervous system effects have been produced by a compound, DFP, which to date, has been shown to have no other important action than its ability to destroy ChE. The production of these central nervous system effects by a compound which has been shown to inhibit ChE within the brain suggests that the acetylcholine cycle does play a positive, though as yet undefined, role in central neural function.

Compatible observations that have been reported in man include the occurrence of electroencephalic abnormalities following the intravenous administration of acetylcholine to epileptic subjects (11), and the occurrence of generalised convulsions following the intracisternal and intravenous administration of acetylcholine (4, 5).

### SUMMARY

1. The administration of DFP, a potent anticholinesterase drug, to human subjects caused central nervous system symptoms and elec-

troencephalographic changes which indicated increased neural activity.

2. The electroencephalographic changes caused by DFP consisted of an increase in the potential, frequency, and irregularity of rhythm, and the intermittent appearance of abnormal waves similar to those seen in patients with grand mal epilepsy.

3. These electroencephalographic changes were promptly reversed by atropine and were not affected by neostigmine or curare. The central nervous system symptoms were inhibited to some degree by atropine.

4. Atropine caused some reduction in the electrical activity of the brain in one-third to one-half of the normal and epileptic subjects studied. The effect was similar to, but not as marked as, the effect of atropine on the electroencephalographic changes due to DFP.

5. The administration of DFP to two subjects with upper motor neurone lesions produced evidence of increased spinal cord activity and of spontaneous discharges within the spinal cord below the level of the lesions.

6. These central nervous system effects of DFP, their inhibition by atropine, and their increase in the presence of an upper motor neurone lesion suggest that the acetylcholine cycle plays a role in central neural function.

## REFERENCES

1. CANNON, W. B., AND HAIMOVICI, H.: The sensitization of motoneurons by partial denervation. *Amer. J. Physiol.*, 1929, 126: 731.
2. DARROW, C. W., PATHMAN, J. H., AND KRONENBERG, G.: Improvement of the electroencephalogram by atropine. *Fed. Proc. Amer. Soc. exp. Biol.*, 1945, 4: 16.
3. FELDBERG, W.: Present views on the mode of action of acetylcholine in the central nervous system. *Physiol. Rev.*, 1945, 25: 573.
4. FIAMBERTI, A. M.: Seizure of epileptic nature produced by the suboccipital administration of vasodilator substance. *Rev. sper. di freniat.*, 1937, 61: 834.
5. FIAMBERTI, A. M.: On the mechanism of therapeutic action of the "vascular storm" produced by choline derivatives. *Gior. di psichiat. e di neuropat.*, 1939, 67: 270.
6. GROB, D., LILIENTHAL, J. L., JR., HARVEY, A. M., AND JONES, B. F.: The administration of DFP to man, I. *Bull. Johns Hopkins Hosp.*, 1947, 81: 217.
7. GROB, D., LILIENTHAL, J. L., JR., AND HARVEY, A. M.: The administration of DFP to man, II. *Bull. Johns Hopkins Hosp.*, 1947, 81: 245.



8. KOELLE, G. B., AND GILMAN, A.: The relationship between cholinesterase inhibition and the pharmacological action of DFP. *J. Pharmacol.*, 1946, **87**: 421.
9. LANARI, A.: The action of intra-arterial acetylcholine in certain conditions of the nervous system and of the muscles. *C. R. Soc. Biol., Paris*, 1936, **123**: 1090.
10. MAZUR, A., AND BODANSKY, O.: The mechanism of in vitro and in vivo inhibition of cholinesterase activity by DFP. *J. biol. Chem.*, 1946, **163**: 261.
11. WILLIAMS, D.: Effect of cholin-like substances on cerebral electrical discharges in epilepsy. *J. Neurol. and Psychiat.*, 1941, **4**: 33.

# THE ADMINISTRATION OF DI-ISOPROPYL FLUOROPHOSPHATE TO MAN

## IV. THE EFFECTS ON NEUROMUSCULAR FUNCTION IN NORMAL SUBJECTS AND IN MYASTHENIA GRAVIS<sup>1</sup>

A. M. HARVEY, J. L. LILIENTHAL, JR., D. GROB, B. F. JONES, AND S. A.  
TALBOT

*From the Physiological Division, Department of Medicine, The Johns Hopkins University  
and Hospital, Baltimore, Maryland*

The peculiar ability of di-isopropyl fluorophosphate (DFP) to inactivate cholinesterase (ChE) irreversibly provides a unique pharmacological tool for an analysis of certain cholinergic phenomena occurring during neuromuscular function, whether normal or disturbed. This paper reports the result of such a study in 15 normal subjects and in seven patients with myasthenia gravis. In addition, an assay has been made of the value of DFP in the treatment of myasthenia gravis in a group of ten patients with this disease in varying degrees of severity.<sup>2</sup>

### METHODS

The injection of drugs under study followed the general plan described previously (9). Repeated injections were facilitated by introducing an inlying needle into the brachial artery. DFP was dissolved in physiological saline in a concentration of 1 mg. per ml. Neostigmine (Prostigmine, Hoffmann-LaRoche) was injected in an aqueous solution containing 0.5 mg. per ml. Curare (Intocostin, Squibb) was injected in an aqueous solution containing 10 or 20 units per ml.

Grip strength was measured by a hand dynamometer (Stoelting). The electromyogram of the abductor of the 5th finger was recorded by the method of Harvey and Masland (7).

### THE INTRA-ARTERIAL INJECTION OF DFP IN NORMAL SUBJECTS

DFP was injected into the brachial artery of 15 normal individuals in doses ranging from 0.5 to 2 mg. Measurable effects on neuromuscular conduction were observed after the smallest of these doses and increasing effects were noted following the larger doses. Atropine,

<sup>1</sup> Work performed under a contract between the Medical Division, Chemical Corps, U. S. Army, and the Johns Hopkins University.

<sup>2</sup> A preliminary abstract of this paper appeared in Fed. Proc., 1946, 5: 182.

which was administered hypodermically prior to each injection in doses of 0.5 to 1.0 mg., did not influence the effect of DFP on neuromuscular conduction, and while it may have diminished sweating in the arm below the point of injection, in no case did it prevent this entirely.

Numerous spontaneous fasciculations appeared in the muscles of the injected area, having the same characteristics as those which follow the injection of neostigmine and those appearing in patients with

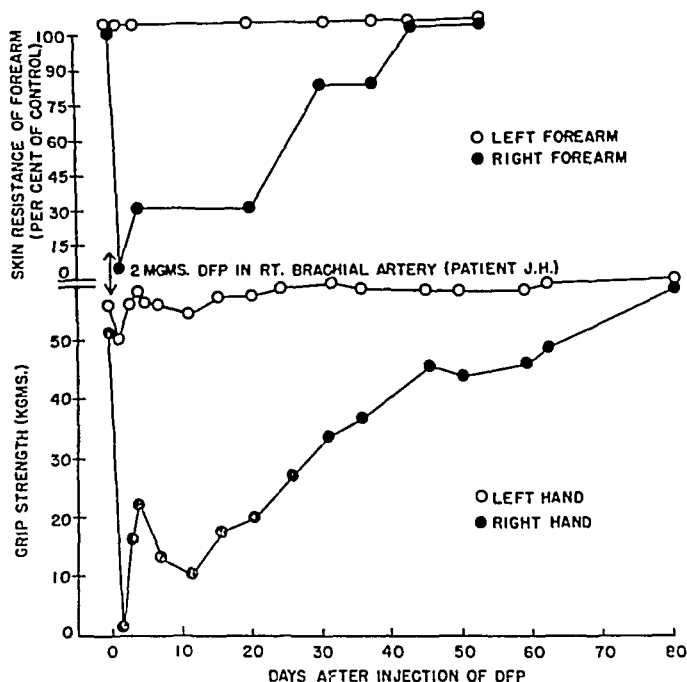


FIG. 1. The time course of the recovery in muscle power and skin resistance in a normal subject after the injection of 2 mg. of DFP into the right brachial artery.

chronic lower motor neurone disease. The subjects usually complained of coldness in the arm and hand below the point of injection.

Pronounced motor weakness was noted in the muscles of the injected area, its degree being proportional to the dosage of DFP. This weakness was not always uniform in distribution; for example, in one experiment the injection produced marked weakness of extension at the wrist and fingers which greatly overshadowed the weakness present in the flexor muscles in the forearm and hand. Figure 1 shows the

comparative strength of grip in the injected and uninjected hand after the administration of 2 mg. of DFP in the brachial artery. It may be noted that there was a very pronounced paresis of the muscles of the injected hand, and that strength returned slowly over a period of 11 weeks. This was accompanied by a decrease in skin resistance due to increased sweating with gradual return to normal over a period of six weeks. After a smaller dose of DFP (0.5 mg.) there was only slight reduction in strength and increase in sweating, and by the following day the only observable effect was easy fatigue of the muscles of the hand.

Electromyographic studies demonstrated the similarity of the effects of intra-arterial DFP and neostigmine on neuromuscular function. There was no change in the voltage of the muscle potential in response to a single maximal motor nerve stimulus. However, this response became repetitive in nature, and instead of the usual diphasic potential the initial spike was followed by a series of smaller potentials which showed progressive decline in voltage (Figures 2 and 3). Table 1 shows the voltage of the successive spikes and their time course. The response to a second stimulus, delivered within a period of 80 msec. after the first, did not show any evidence of the repetitive discharge. Other observations with various doses of DFP indicated that this period of suppression was proportional to the amount of repetition in the first response.

When two maximal nerve stimuli were delivered the voltage of the second muscle action potential was reduced. This reduction of voltage was greatest when the intervals between the two stimuli were short and lessened as the interval was increased. Table 2 shows the interval-potential relationship of this depression of the second of two responses at various time intervals after the intra-arterial injection of DFP. The voltage of the initial response is expressed as 100 per cent and the potential of the second response is recorded in terms of per cent of the first.

It is of interest that the maximal effect of the DFP was not reached immediately, but developed in 30 to 60 minutes. Except for the duration of the effect, which was found to be as long as 11 weeks in one patient, the changes observed in neuromuscular function were essentially similar to those observed after neostigmine where the maximal depression of function in the normal subject is seen within five minutes, and disappears within 20 to 45 minutes (9).

The changes seen in responses to repetitive nerve stimuli were also very striking. In Table 3 the successive responses are expressed in terms of per cent of the potential set up by the initial stimulus, the voltage of which is 100 per cent. The interval between the successive stimuli was 20 msec. This table demonstrates that after the injection



FIG. 2. Normal subject. Muscle action potentials in response to supramaximal motor nerve stimuli: 1 and 2: Responses to 2 nerve stimuli at intervals of 30 and 80 msec. before DFP. 4 and 5: Responses to 2 nerve stimuli 30 min. after the injection of 1.0 mg. of DFP into the brachial artery. 3 and 6: Train of 3 responses before and after DFP (stimulation rate approx. 30 per sec.). 7: Two responses after DFP at higher amplification to show the nature of the repetitive discharge more clearly. Voltage of the initial potential in (1) 8 mV. This figure illustrates the repetitive character of the initial response, the depressed voltage of the second response, and the varying responses to a train of stimuli, following the intra-arterial administration of DFP.

of DFP the second response is greatly depressed, but the third response shows a remarkable recovery. An example of this phenomenon is seen in Figure 3. This is in distinct contrast to the train of responses observed after the intra-arterial injection of neostigmine where there is a progressive decline in voltage of the successive potentials (Table 3). The significance of this difference in the effect of the two drugs on the

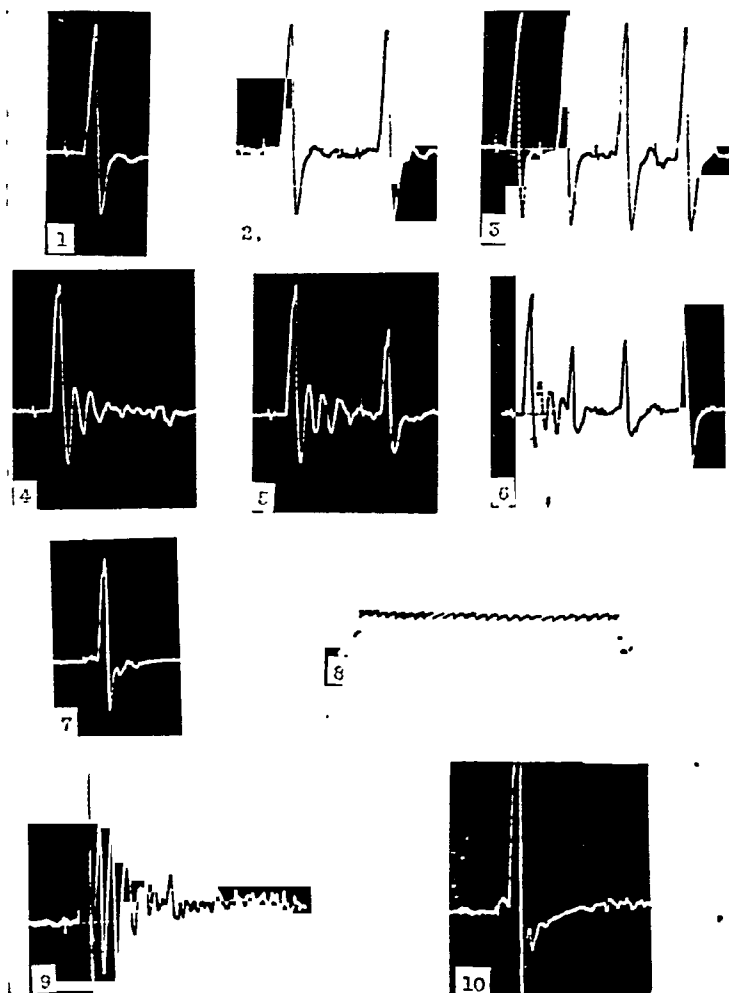


FIG. 3. The electromyogram of a normal subject before and after the intra-arterial injection of 0.5 mg. DFP and 9 units curare. 1, 2, and 3: Control. 4, 5, and 6: After DFP. 9: Amplified record corresponding to 4 to show repetitive response. 8: Calibration: 4mV. and 5 msec. 7: After DFP followed by curare. 10: Amplified record corresponding to 7 to show disappearance of repetitive response.

responses to a train of stimuli is not apparent from the observations made in the experiments described here.

In the normal subjects who received intra-arterial neostigmine a

TABLE 1

*The muscle response to a single maximal motor nerve stimulus following the intra arterial injection of DFP in a normal subject*

	INITIAL SPIKE	REPETITIVE RESPONSES		
		1st	2nd	3rd
Voltage	6 mV.	3.4 mV.	1.8 mV.	0.52 mV.
Time of appearance	0	6.7 msec.	18 msec.	30 msec.

TABLE 2

*The depression of the second of two responses of the muscle to maximal motor nerve stimuli in a normal subject*

Figures represent voltage of second response expressed as per cent of initial response

INTERVAL BETWEEN STIMULI (MSEC)	BEFORE DFP	TIME AFTER INJECTION OF DFP			
		5 min	70-100 min	20 hrs	65 hrs
10- 19	99	56		41	
20- 29			37	52	64
30- 39	97	66		74	76
40- 49	98	75	53	77	
50- 59					85
60- 69	97	88		82	
70- 79			66	84	85
80- 89		90			88
90- 99	98		69		
100-110			69		

TABLE 3

*Muscle responses to repetitive maximal motor nerve stimuli at a rate of 50 per sec. in a normal subject*

Figures represent per cent of voltage of response to initial stimulus

RESPONSE NO	TIME AFTER DFP INJECTION (INTRA-ARTERIAL)					TIME AFTER NEOSTIGMINE INJECTION (INTRA-ARTERIAL)
	0	10 min	70 min	20 hrs	65 hrs.	20 min.
1	100	100	100	100	100	100
2	97	50	32	24	46	74
3	97	59	45	58	74	45
4	96	72	53	66	59	26

spread of the effect throughout most of the muscles of the body was noted frequently within five minutes after release of the cuff, as indicated by the appearance of generalised fasciculations. No such generalised effects have been observed following even the largest doses of DFP injected intra-arterially. All of the manifestations have remained localised to the area distal to the site of intra-arterial injection.

Fasciculations were usually the first of the various neuromuscular manifestations to disappear. Thus, in the above patient who received 2 mg. of DFP, fasciculations were noted for only nine days. However, occasionally a few fasciculations have appeared following a dose of DFP which produced little or no motor weakness and in a few cases persisted after motor power had returned to normal. The repetitive response to a single stimulus usually was demonstrable for a longer period than the motor weakness. In one instance, there was motor weakness for only a few hours after the injection of 0.5 mg. of DFP while the repetitive discharge persisted for five days.

#### THE EFFECT OF CURARE UPON THE CHANGES PRODUCED BY THE INTRA-ARTERIAL INJECTION OF DFP IN NORMAL SUBJECTS

The effect of intra-arterial curare is demonstrated by the accompanying illustrations of the electromyograms of a normal male who received 0.5 mg. of DFP in the right brachial artery, and developed in the injected arm transient motor weakness, numerous fasciculations and diffuse sweating (Figure 3). The electromyographic changes which followed the injection of DFP were as described before: repetitiveness, depression of the second response to two stimuli, and the varying responses to a train of stimuli. Thirty-five minutes after the injection of DFP, nine units of curare were injected into the same artery. A bright flush appeared over the arm and there developed moderate, generalised swelling of the extremity with wheal formation (3). The repetitive response to single stimuli disappeared completely, and had not returned 30 minutes later. The voltage of the response to a single stimulus was reduced immediately following curare, but during the period of observation, slowly returned to the normal voltage. When virtually normal voltage had returned the repetitive response was still absent.



THE INTRA-ARTERIAL INJECTION OF DFP IN PATIENTS  
WITH MYASTHENIA GRAVIS

DFP has been injected intra-arterially in seven patients with myasthenia gravis on 14 occasions in doses ranging from 0.5 to 1.5 mg. The response was uniform in all of these patients and is well exemplified in the following experiment: After a period of 72 hours without neostigmine, the patient was given an injection of 0.5 mg. of DFP into the right brachial artery. Almost immediately profuse sweating appeared below the level of injection and local muscular power increased rapidly, reaching its maximum in approximately 15 minutes. No fasciculations were observed at any time following the injection. Electromyographic studies, made prior to the injection of DFP in this individual, showed the typical depression of the second of two action potentials in response to a pair of maximal motor nerve stimuli (8). When a train of four stimuli were applied to the nerve at intervals of 20 msec. there was a progressive decrease in size of muscle action potentials even during the period when the patient received 450 mg. of neostigmine orally each day. Table 4 shows the responses to such a train of stimuli during various stages of treatment. The character of the change in the electromyogram after the intra-arterial injection of DFP was similar to that which followed the injection of neostigmine (Figure 4). The improvement reached its maximum degree between one to two hours after the injection, but in this instance the dose apparently was insufficient to restore neuromuscular conduction to normal.

Table 5 shows the change in the successive responses to a train of stimuli at various time intervals after the intra-arterial injection of 0.5 mg. of DFP. Nine days after the injection of the DFP the dynamometer test showed persistent increased strength in the injected hand, 15 kg. as compared to a grip of 8 kg. before the injection. The control or uninjected hand had a grip strength of 8 kg. During this period there was persistent increase in sweating below the point of injection, and the patient stated that the hand on that side usually felt colder than the uninjected hand. It must be emphasised that at no time was there any increase in muscular strength elsewhere than distal to the level of intra-arterial injection. This is in contrast to the effect of neostigmine in myasthenia gravis where an intra-arterial injection of this drug produces a similar great increase in strength locally, but,

in addition, a generalised increase in motor power after the release of the cuff. On the third day after the injection of DFP the patient was

TABLE 4

*Muscle responses to repetitive maximal motor nerve stimuli at a rate of 50 per sec. in a myasthenic subject during various stages of treatment*

Figures represent voltage of response expressed as per cent of initial response

RESPONSE NUMBER	450 MG. NEOST. ORALLY IN 24 HRS.	OFF NEOST. 72 HRS.	15 MIN. AFTER 1.5 MG. NEOSTIG. INTRA-ART.
1	100	100	100
2	90	54	100
3	64	33	100
4	58	29	100

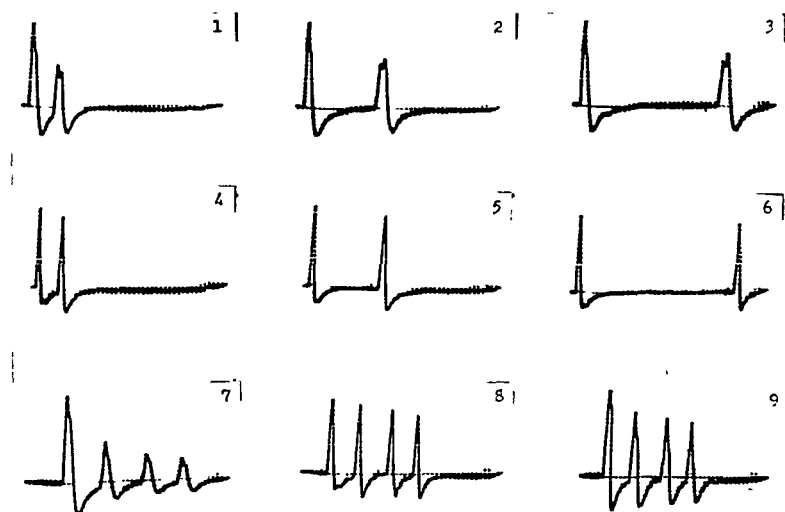


FIG. 4. Myasthenia gravis. Muscle action potentials in response to maximal motor nerve stimulation before and after the injection of 0.5 mg. DFP into the brachial artery. 1, 2, and 3: Before DFP. Responses to two nerve stimuli at various time intervals. 4, 5, and 6: Similar records 24 hours after DFP. 7: Train of four stimuli (approx. 40 per sec.) before DFP. 8 and 9: Similar trains at 24 and 48 hours after DFP.

given 1.5 mg. of neostigmine in the opposite (left) brachial artery to compare the maximum effects of the two drugs and to observe the time

relationship of the action of the two drugs when given by this route. Table 6 shows the grip strength of the two hands before and during this experiment. This maximum response reached by the right hand after

TABLE 5

*Muscle responses to repetitive maximal motor nerve stimuli at a rate of 50 per sec. in a myasthenic subject before and at various time intervals after the intra-arterial injection of 0.5 mg. of DFP*

Figures represent voltage of response expressed as per cent of initial response

RESPONSE	BEFORE DFP	15 MIN. AFTER	40 MIN.	4 HRS.	24 HRS.	96 HRS.
1	100	100	100	100	100	100
2	54	75	66	95	85	72
3	33	63	55	89	75	53
4	29	54	46	80	70	50

TABLE 6

*The effect on the grip strength of a myasthenic subject of intra-arterial neostigmine administered 3 days after intra-arterial DFP*

	GRIP STRENGTH (DYNAMOMETER)	
	Right hand (kg.)	Left hand (kg.)
(3 days after DFP (right artery))	18	8
Time after injection of 1.5 mg. Neostig (left artery) (minutes)		
1	35	30
4	43	30
9	46	37
21	46	48
68	45	45
103	35	35
123	35	30
163	36	25
213	29	15
238	29	13
258	29	12
350	29	10

the injection of DFP was 44 kg. It is interesting that although the neostigmine was injected into the left brachial artery the muscles in the right arm, which had been injected previously with DFP, responded

more promptly and showed a more prolonged effect. However, the maximum degree of improvement was the same on the two sides.

One patient with extreme myasthenia gravis was studied with particular reference to the comparative effects of intra-arterial DFP and neostigmine. Before either drug was administered the voltage of the action potentials was low. After the intra-arterial injection of 1.5 mg. of neostigmine the voltage of the response to a single stimulus was doubled, and the responses to a train improved. The same degree of improvement was recorded on another occasion after the intra-arterial injection of 0.8 mg. of DFP.

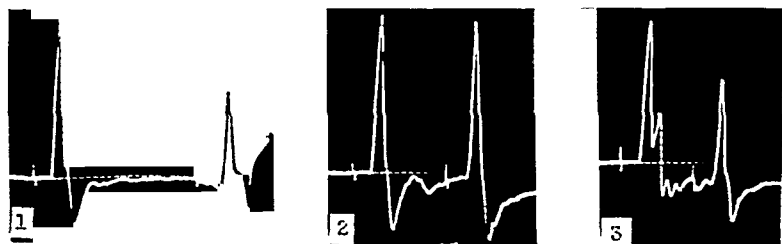


FIG. 5. Electromyogram of a myasthenic subject after DFP and after subsequent neostigmine. 1: Control record; no medication for 7 hours. 2: Repair of neuromuscular condition after 0.5 mg. of DFP intra-arterially. 3: Deterioration of neuromuscular conduction following 0.5 mg. neostigmine (29 minutes after record 2) with development of repetitive discharge as seen in the first of the two responses.

#### THE INTERRELATIONSHIPS OF DFP AND NEOSTIGMINE

##### *DFP Followed by Neostigmine*

A patient with severe myasthenia gravis which involved the bulbar musculature predominantly, but with considerable weakness of the hands, received 0.5 mg. of DFP intra-arterially with subsequent improvement of neuromuscular conduction as measured by the response to a train of four stimuli. Nineteen minutes later he received 0.5 mg. of neostigmine in the same artery. Electromyographic measurements of neuromuscular conduction showed impairment, as evidenced by a depression in the voltage of the second response to a pair of stimuli and by the appearance of repetitive response to the first stimulus (Figure 5).

Thus, in a muscle exhibiting a typical myasthenic response, neuro-

muscular conduction was repaired by intra-arterial DFP; a subsequent injection of neostigmine then depressed neuromuscular conduction and induced repetitive discharge in a fashion similar to its effect on normal muscle. This train of events, which represents the first observation of this nature in our experience, might be interpreted to result from the additive anti-cholinesterase effects of an initial dose of DFP followed by a subsequent dose of neostigmine which permitted the development of a paralysing concentration of acetylcholine at the neuromuscular junction. This indicates that neostigmine administered after DFP can produce a summated anti-cholinesterase effect.

### *Neostigmine Followed by DFP*

In the experiments in which DFP was injected intra-arterially it was noted that the effect was appreciably less in patients who had received neostigmine shortly before the injection of the DFP. Also, it was observed that when DFP was given intramuscularly to a myasthenic patient who was receiving his regular neostigmine medication the effect of the DFP was reduced. These observations suggested that neostigmine inhibited the effect of DFP. To analyse this relationship in more detail, two patients with myasthenia gravis were given on five occasions an injection of DFP into the brachial artery at a time when they had received no neostigmine during the preceding 24 hours. Injections of DFP were repeated after an interval of one week in the opposite brachial artery at a moment when the patients were at the point of maximal benefit from an intramuscular injection of 2 mg. of neostigmine. Figure 6 demonstrates that when the patient had had a recent injection of neostigmine the intra-arterial injection of DFP did not produce the lasting increase in muscular power which followed the administration of DFP alone. The results were uniform in all of these experiments. These observations suggest that neostigmine and DFP compete for a common site of action, and that when neostigmine is present at this site it blocks the action of DFP. In order to exclude the possibility that this effect may have been due to the residual presence of DFP the blocking experiment was done on several occasions in patients who had never received a previous injection of DFP. The results were entirely similar to those in which the patient had received a DFP injection one week prior to the blocking experiment. Addi-

tional observations showed that the presence of DFP does not block the action of subsequent injections of this drug; in one patient DFP injections were made into the same artery at an interval of three days and the second injection produced the same maximum response as that which had resulted originally.

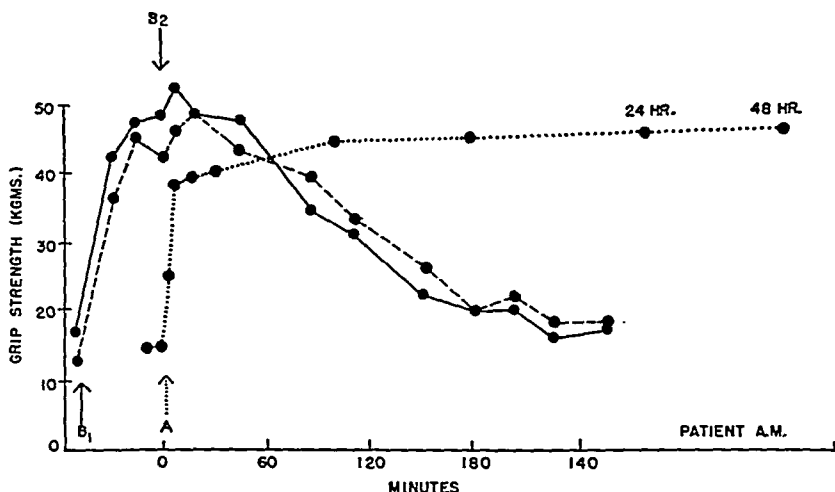


FIG. 6. The blocking effect of neostigmine on the response of the myasthenic muscle to DFP injected intra-arterially. . . . Right grip showing the prolonged response of the muscles to DFP injected into the right brachial artery at A when the patient had had no neostigmine for 24 hours. ——— Left grip, - - - Right grip in an experiment done one week later showing the response to 2 mg. of neostigmine administered intramuscularly at B<sup>1</sup>. At B<sup>2</sup> 0.65 mg. of DFP was injected into the left brachial artery while the neostigmine effect was at its height. The strength in the two hands returned to their original levels at the same time, in contrast to the prolonged effect of DFP in the absence of neostigmine.

#### THE TREATMENT OF PATIENTS WITH MYASTHENIA GRAVIS WITH DFP

Ten patients with myasthenia gravis have been treated for periods of time varying from a few weeks to several months with intramuscular injections of DFP in oil. These patients were selected to represent most of the variations observed in the clinical picture of myasthenia gravis, with cases ranging from early and very mild forms of the disease to patients in whom the disease had been present a long time and was

very severe. As shown in Table 7 the distribution in some instances was primarily bulbar while in others the muscles of the trunk and extremities were predominantly involved. The cases also presented a fairly inclusive picture in relation to previous treatment. One individual had never before received any drug for the treatment of myasthenia gravis while others had received neostigmine for a short period of time and, in many, treatment with this drug had been con-

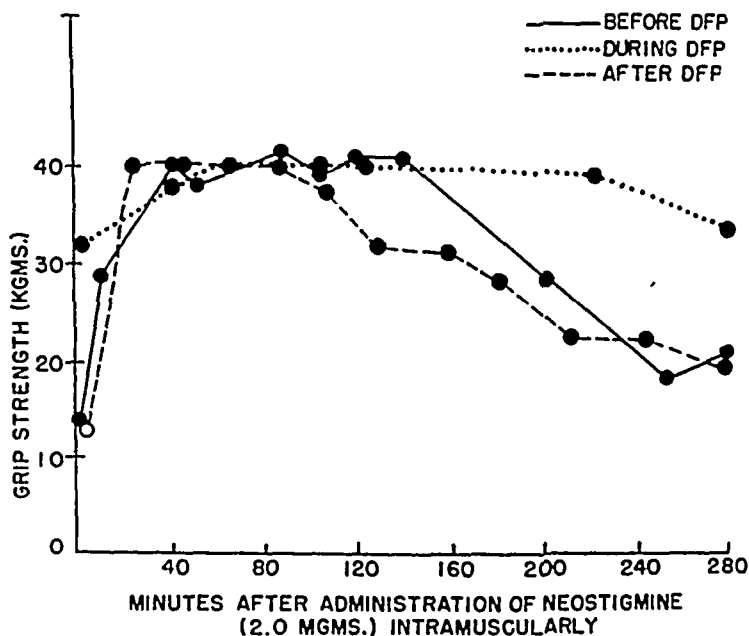


FIG. 7. The effect of neostigmine in a patient with myasthenia gravis before, during, and after the administration of DFP. Although the basal strength was greater during DFP therapy, the maximum response to the neostigmine was the same.

stant over a number of years. In four individuals thymectomy had been performed. In two of these this was done before DFP treatment was given, but in two others the drug was administered both before and after the removal of the thymus gland. Because of the recognised emotional states which may occasionally simulate this disease, great care was taken to establish the diagnosis beyond question in each case. A variety of facts were gathered for this purpose. Each patient was

examined by several physicians, and agreement as to the diagnosis was uniform. A variety of other examinations were carried out to substantiate the diagnosis, including therapeutic tests with neostigmine, administration of quinine, intra-arterial injection of neostigmine, and detailed electromyographic studies following supra-maximal stimulation of the motor nerve. No individual was included in this study unless these various tests and examinations gave incontrovertible

TABLE 7  
*Description of cases of myasthenia gravis treated with DFP*

SUBJECT	AGE	SEX	COLOR	DISTRIBUTION OF WEAKNESS		SEVERITY	DURATION (YEARS)	HISTORY OF REMISSION	TREATMENT WITH NEOSTIGMINE (MG.)		THYMECTOMY	REMARKS
				Oculo-bulbar	Peri-pheral				Oral	Hypo-der-mal		
B. S.	23	F	W	+++	+++	+++	14	0	360	1.0*	0	
A. M.	30	M	C	+	++++	+++	1	0	270	1.0*	+	Thymectomy after treatment with DFP
J. H.	23	M	W	++++	++	+++	1	0	270	4.0	0	
R. N.	30	F	W	++++	++	+++	5	0	225	2.0	0	
R. P.	34	M	C	++++	++	+++	12	Partial after thymectomy	150	1.0*	+	Thymectomy before treatment with DFP
B. W.	18	F	C	+++	++	+++	6	Partial after thymectomy	150	1.0*	+	Thymectomy before treatment with DFP
J. W.	30	F	W	+++	++	+++	1½	0	120	0	0	
L. C.	24	F	W	0	+++	++	7	0	90	0	0	
F. T.	10	F	C	++	+	++	1	0	90	0.5*	0	No treatment with neostigmine before trial on DFP
C. P.	41	M	W	++++	++++	++++	1	0	0	20	+	Thymectomy after treatment with DFP

\* Rarely.

evidence that the patient had myasthenia gravis. DFP was administered in doses ranging from 0.5 to 2 mg., given intramuscularly in peanut oil, and repeated usually at daily intervals until the cholinesterase had been reduced to the maximum degree possible without the production of unbearable symptoms. When sufficient doses of atropine were given to control symptoms it was possible to reduce the red cell esterase to very low levels. The autonomic and central neural



symptoms which these patients developed as a result of DFP administration were essentially the same as those observed in normal individuals (4). Atropine did not alter the effects of DFP on skeletal muscle.

In general, DFP has not proved to be a promising therapeutic agent for myasthenia gravis. A definite beneficial effect was obtained in each of the ten individuals, but only in the cases with very mild weakness were the patients able to carry on any degree of activity approaching that possible with neostigmine. Symptoms due to the reduction of cholinesterase activity in the central nervous system and gastro-intestinal tract were almost uniformly present in these individuals and with one exception the patients much preferred treatment with neostigmine. In the severe cases, DFP was noted to have a slight effect in enhancing the response to neostigmine, but it was not possible to bring about more than a moderate reduction in neostigmine requirement in cases of this type without doses of DFP which caused undesirable central nervous system and gastro-intestinal symptoms. In the mild and moderately severe cases it was possible to reduce the daily neostigmine requirement to a significant degree. In these patients it was observed that when they were receiving DFP they could reach their maximum possible response to neostigmine with smaller doses of the latter drug. All of the myasthenic patients, after a rest period overnight without medication and tested in a basal state in the morning, were observed to have an increase in their strength and in the variety of activities which they could carry out without medication. However, these improvements in their clinical condition did not usually approach that possible with satisfactory amounts of neostigmine, and it was found that when neostigmine was given in addition to the basal dose of DFP the maximum increase in strength never exceeded that which was possible on neostigmine alone (Figure 7 and Table 8).

Additional evidence of this limitation of the physiological capacity of neuromuscular function was indicated by the fact that with DFP some of these patients could be brought to a maximum degree of strength, beyond which an added dose of neostigmine carried them over into a state of weakness simulating that seen in normal individuals following an excessive dose of an anticholinesterase drug (Figure 5).

In an assessment of the value of DFP it was found that this drug had to be given at a time when the patient was not under the influence

TABLE 8

*The influence of the daily administration of DFP on the cholinesterase activity (plasma and red blood cell), neostigmine requirement, and strength of 10 myasthenic patients*

PATIENT	NO. OF DAYS	DFP AV. DAILY DOSE (MG.)	BASAL CHOLIN- ESTERASE AT END OF EACH PERIOD (%)		NEOSTIGMINE AV. DAILY REQUIRE- MENT (MG. ORAL)	GRIP STRENGTH (KG.)		BASAL CONDITION
			Plasma	RBC		Av. basal last 5 days of each period	Max. after neostig- mine	
B. S.	12	0	100	100	360	8	33	Unable to get out of bed or swallow food. General strength moderately improved; could do above. Returned to original condition within two weeks.
	37	0.44	9	23	219	21	34	
	10	0	52	32	261	21	35	
A. M.	22	0	100	100	270	13	52	Unable to lift head off pillow or roll over in bed. General strength moderately improved; could do above. Returned to original condition within one week.
	10	1.00	10	42	180	28	54	
	10	0	58	52	204	13	54	
J. H.	7	0	100	100	261	15	48	Unable to get out of bed or swallow liquid. General strength moderately improved; could do above. Returned to original condition in two weeks.
	33	1.04	10	22	109	30	51	
	15	0	85	31	137	17	42	
R. N.	37	0	100	100	223	8	29	Unable to get out of bed or swallow liquid. General strength moderately improved; could do above. Returned to original condition within two weeks.
	25	1.25	10	22	106	15	30	
	17	0	79	34	129	9	30	

TABLE 8—*Continued*

PATIENT	NO. OF DAYS	DFP AV. DAILY DOSE (MG.)	BASAL CHOLIN-ESTERASE AT END OF EACH PERIOD (%)		NEOSTIGMINE AV. DAILY REQUIREMENT (MG. ORAL)	GRIP STRENGTH (KG.)		BASAL CONDITION
			Plasma	RBC		Av. basal last 5 days of each period	Max. after neostigmine	
R. P.	11	0	100	100	131	36	66	Ptosis, diplopia, facial weakness, difficulty swallowing, easy fatigue.
	64	0.69	7	3	0	46	52	General strength and above symptoms moderately improved.
	34	0	85	30	36	34	52	Returned to original conditions within three weeks.
B. W.	16	0	100	100	124	8	25	Unable to get out of bed; ptosis, facial weakness; difficulty swallowing.
	56	0.66	8	48	89/0	30	32	General strength and above symptoms markedly improved.
	8	0						On eighth day died outside of hospital in sudden exacerbation of weakness.
J. W.	29	0	100	100	119	24	39	Ptosis, diplopia, facial weakness, unable to swallow food.
	33	1.13	6	15	92	34	40	General strength and above symptoms moderately improved.
	21	0	77	41	91	28	42	Strength improved over original condition after four weeks.

TABLE 8—*Concluded*

PATIENT	NO. OF DAYS	DFP AV. DAILY DOSE (MG.)	BASAL CHOLIN-ESTERASE AT END OF EACH PERIOD (%)		NEOSTIGMINE AV. DAILY REQUIREMENT (MG. ORAL)	GRIP STRENGTH (KG.)		BASAL CONDITION
			Plasma	RBC		Av. basal last 5 days of each period	Max. after neostigmine	
L. C.	15	0	100	100	69	15	39	Unable to get out of bed.
	8	1.69	9	46	49	30	40	General strength slightly improved; could do above.
	20	0	64	54	67	16	40	Returned to original condition within one week.
F. T.	11	0	100	100	0	12		Ptosis, diplopia, easy fatigue.
	11	0.42	21	17	0	20	22	General strength and above symptoms moderately improved.
	11	0	46	36	0	16	23	Returned to original condition within one week.
C. P.	18	0	100	100	20 (mg. hypo)	0	22	Unable to lift head off pillow or swallow liquid; ptosis, diplopia.
	27	0.46	13	41	17 (mg. hypo)	3	15	General strength and above symptoms slightly improved.
	8	0	37	51	20 (mg. hypo)	0		Returned to original condition within one week.

of neostigmine; it was administered, therefore, early in the morning after the patient had passed the night without medication. In this way the possible competition of these two drugs for a similar site of action could be excluded as a factor affecting the clinical response to DFP.

All of these patients were followed carefully after the cessation of DFP treatment and in most of them there was a gradual decline in basal muscular strength, the details of which can be seen in Table 8. Plasma and red blood cell cholinesterase activity rose gradually to the previous normal levels. The daily neostigmine requirement which was reduced during the period of DFP treatment returned gradually to its previous level. There was no demonstrable difference in the response to DFP before and after thymectomy.

It is noteworthy that following cessation of DFP treatment three of the patients in this series died, all with essentially the same clinical picture. In each instance the patient developed an infection of the upper respiratory tract which was followed by rapid increase in weakness, acute respiratory distress and death in a few hours. The first patient (B. W.) had received 37 mg. of DFP in 56 days, had had no DFP for a period of eight days, had complained of no symptoms which could be attributable to the anticholinesterase activity of this drug, and had been visiting the hospital daily for check-up. The morning of her death, which was the ninth day after the last dose of DFP, she arose, took 30 mg. of neostigmine by mouth and started for the hospital in apparently good health except for a mild cold. While on the way she suddenly became extremely short of breath, and was immediately sent to the hospital where she arrived unconscious. Efforts to revive her failed, and she died within a few hours.

The second patient (R. P.) had a rather severe form of the disease which had been characterised over a period of years by numerous spontaneous remissions and relapses following infections of the upper respiratory tract. He was treated for a period of two months with DFP (total dose 44 mg.) and was in the hospital for observation after the cessation of this drug during the period of readjustment to neostigmine. He remained under observation for eight weeks during which time he showed wide variations in strength and in neostigmine requirements. During this period he contracted an upper respiratory tract infection which made difficult the control of his myasthenia gravis. He was discharged from the hospital at his own request 55 days after the last dose of DFP on a daily neostigmine dosage of 150 mg. by mouth, amplified by occasional hypodermic injections of 1 mg. for nocturnal choking spells. During the following ten days his strength

declined, and he required frequent emergency doses of neostigmine. He was urged to return to the hospital, but refused. On the ninth day after discharge and the 64th day after the last dose of DFP, he developed a sudden exacerbation of his weakness with choking, and died on the way to the hospital.<sup>3</sup>

The third patient (J. W.) was a 30 year old married female who received DFP in oil for 33 days (in doses varying from 1 to 1.5 mg.) for a total of 37 mg. She had a good response to this drug, with a moderate increase in basal strength, and some decrease in neostigmine requirement. She had no other symptoms attributable to the DFP. She was discharged from the hospital 34 days after her last dose of DFP, at which time she had undergone what was considered a slight remission of her disease, as her basal strength was somewhat improved. Almost five months after her last dose of DFP she developed a mild infection of the upper respiratory tract, became rapidly weaker, and died of respiratory paralysis before she could be brought to the hospital.

#### DISCUSSION

##### *Comparison of Action of DFP and Neostigmine*

DFP and neostigmine both inhibit ChE, the former irreversibly, the latter reversibly. This difference in their actions becomes apparent when the duration of effects following intra-arterial injection are compared. The local sweating, weakness and fasciculations disappear within one hour after neostigmine; after DFP these effects may persist for hours, days or weeks depending upon the amount injected. The rate of return of normal function probably reflects the rate of local regeneration of ChE.

The injection of either DFP or neostigmine converts the single diphasic electrical response of normal muscle to a single shock stimulation of the motor nerve into a repetitive response. This evidence of hyperexcitability of the neuromuscular unit is observed only after the first stimulus; a second stimulus presented within less than 110 msec. evokes a reduced response which is not repetitive. This sequence of hyperexcitability and depression is consonant with the common anti-

\* Autopsies performed on patients B. W. and R. P. revealed lobular pneumonia sufficient in extent to explain sudden exitus in a patient with myasthenia gravis (4).

cholinesterase action of these agents, and has been interpreted to indicate, first, persistence at the nerve endings of acetylcholine and, then, accumulation of acetylcholine to paralysing concentrations (9).

Eccles, Katz and Kuffler (2) in a study of the effect of another anticholinesterase agent, eserine, on the myoneural junction, attributed the repetitive discharge and neuromuscular block to the prolongation of the endplate potential which eserine produces. However, this interpretation of the events occurring at the neuromuscular junction after treatment with eserine could not be applied without modification to the events occurring after injection of DFP. In Figures 2 and 3 it will be noted that the second response to a train exhibits the greatest depression and that although the subsequent responses do not become repetitive they do regain some lost potential. This demonstration of recovery of neuromuscular transmission during a train of stimuli cannot be explained adequately at present.

Another difference between the actions of DFP and neostigmine injected intra-arterially was noted in the consistent localisation of effects to the injected arm after DFP in contrast to the generalised effects which followed neostigmine. This difference was especially marked in patients with myasthenia gravis where DFP produced a local increase in strength in the injected arm but virtually none elsewhere, in contrast to the effects of neostigmine which are generalised regardless of the route of administration. The available evidence furnishes no adequate explanation for this phenomenon, although known differences in the kinetics of reaction of these agents with cholinesterases, and in their solubilities, may play important rôles.

The blocking of DFP effect by prior administration of neostigmine may well be the result of different modes of reaction of these agents with ChE. It may be assumed that after the intra-arterial injection of neostigmine a major portion of the ChE in the arm is combined with neostigmine, and that this ChE-neostigmine complex is not susceptible to attack by the subsequently injected DFP which is then destroyed by fluorophosphatase. Then, presumably, in the course of the next hour, the ChE is released from its combination with neostigmine into an environment which has been freed from DFP. This phenomenon has been noted *in vitro* (10), and *in vivo* in the blocking of DFP action

on the gut (5) and by the protective action of physostigmine against the toxic effects of DFP in cats (11).

If these drugs are injected in the reverse order, DFP followed by neostigmine, the effects are summed rather than opposed. In this case it is probable that the remaining ChE which has not been destroyed by DFP is inhibited by neostigmine. In the example of this additive effect which has been described above a chance combination of doses of these agents evoked in a patient with severe myasthenia gravis a paralysing response to neostigmine. The sequence of events suggests that the combined effects permitted a paralysing concentration of acetylcholine to accumulate at the neuromuscular junction in an instance of myasthenia gravis. This "normal" response to neostigmine was only partial, however, because spontaneous fasciculations did not occur.

The characterisation of phenomena occurring at the neuromuscular junction in terms of known acetylcholine-cholinesterase relationships has proved extremely useful in many attempts to interpret the effects of drugs and disease. Hitherto these interpretations have been based on the assumption that the effects of neostigmine were limited to an inhibition of ChE. The recent report of a direct action of neostigmine on skeletal muscle may require that these earlier interpretations be modified (12).

Curare injected after the DFP effects were well established produced some noteworthy changes. First, there appeared a transient increase in the weakness established by DFP. If the effects of DFP and curare on the neuromuscular junction were the result simply of an excess of acetylcholine (DFP) or lessened response of muscle to acetylcholine (curare) then it might be anticipated that curare would lessen the weakness produced by DFP. This antagonism has been demonstrated and measured experimentally for eserine, another anticholinesterase agent (2). It may well be that the failure of curare to lessen the weakness following DFP in this study was the result of comparative dosage levels; i.e., the amount of curare injected was sufficient to produce neuromuscular block by increasing muscle threshold to acetylcholine to such a degree that any DFP-curare antagonism was masked. A second possibility to be considered is that the preparation of curare



(Intocostrin) used in this experiment may contain impurities which are themselves anticholinesterase in action (6).

That curare did antagonise certain effects of DFP was demonstrated by the suppression of repetitive responses to single stimuli which continued after neuromuscular transmission had returned to normal levels. The counterpart of this effect has been analysed in eserinated experimental animals by Eccles, Katz and Kuffler and explained by their observation that curare reverses the eserine effect of prolonging the endplate potential (2).

### *The Treatment of Myasthenia Gravis with DFP*

The experience in treating myasthenia gravis with DFP has been discouraging in general (1). It is true that definite improvement was observed in several instances but in none was there noted a beneficial effect equal to that afforded by neostigmine. Furthermore the development of unpleasant central neural and gastro-intestinal side-effects precluded vigorous treatment with DFP. When these central neural effects were well established they were not accentuated by the administration of neostigmine, suggesting that their appearance was related to the high lipid solubility of DFP which permitted its penetration into the neuraxis, in contrast to neostigmine which is less soluble in lipids. From these considerations it would seem likely that the general treatment of myasthenia gravis with anticholinesterase agents of this type must await the development of a substance which, by reason of its distribution within the body, will produce maximal effects in striated muscle before it destroys appreciable amounts of ChE in the central nervous system.

We are indebted to Dr. John R. Brewer of Hynson, Westcott, and Dunning, Baltimore, Maryland for his help in ampouling our solutions of DFP.

### SUMMARY

1. In normal subjects intra-arterial injections of DFP produced localised effects distal to the site of injection which were similar in most respects to those produced by neostigmine: weakness, fasciculations, sweating, electromyographic evidence of repetitive response and

depression of neuromuscular transmission. DFP differed from neostigmine in producing its effects more slowly, in the prolonged duration of effects, in the varying responses to a train of stimuli, and in the absence of generalised action.

2. In patients with myasthenia gravis intra-arterial injection of DFP produced local return of motor power which persisted for prolonged periods.

3. Evidence has been adduced to indicate that DFP and neostigmine compete for ChE, and that the formation of a neostigmine-ChE complex will protect the ChE from irreversible inhibition by subsequently injected DFP, thereby blocking the effect of DFP.

4. Treatment of ten patients with myasthenia gravis by DFP resulted in appreciable gain in strength but in no instance was the improvement as great as that obtained with neostigmine. The unpleasant central nervous and gastro-intestinal symptoms produced by DFP precluded its use in amounts sufficient to produce adequate therapeutic effects.

#### REFERENCES

1. COMROE, J. H., JR., TODD, J., GAMMON, G. D., LEOPOLD, I. H., KOELLE, G. B., BODANSKY, O., AND GILMAN, A.: The effect of di-isopropyl fluorophosphate (DFP) upon patients with myasthenia gravis. *Amer. J. Med. Sci.*, 1946, 212: 641.
2. ECCLES, J. C., KATZ, B., AND KUFFLER, S. W. Effect of eserine on neuromuscular transmission. *J. Neurophysiol.*, 1942, 5: 211.
3. GROB, D., LILIENTHAL, J. L., JR., AND HARVEY, A. M.: The vascular response to curare in man: the "histamine" reaction. *Bull. Johns Hopkins Hosp.* 1947, 80: 299.
4. GROB, D., LILIENTHAL, J. L., JR., HARVEY, A. M., AND JONES, B. F.: The administration of di-isopropyl fluorophosphate to man, I. *Bull. Johns Hopkins Hosp.*, 1947, 81: 217.
5. GROB, D., LILIENTHAL, J. L., JR., AND HARVEY, A. M.: The administration of diisopropyl fluorophosphate to man, II. *Bull. Johns Hopkins Hosp.*, 1947, 81: 245.
6. HARRIS, M. M., AND HARRIS, R. S.: Effects in vitro of curare alkaloids and crude curare preparations on "true" and pseudo-cholinesterase activity. *Proc. Soc. exp. Biol.*, 1944, 56: 223.
7. HARVEY, A. M., AND MASLAND, R. L.: A method for the study of neuromuscular transmission in human subjects. *Bull. Johns Hopkins Hosp.*, 1941, 68: 81.

8. HARVEY, A. M., AND MASLAND, R. L.: The electromyogram in myasthenia gravis. Bull. Johns Hopkins Hosp., 1941, 69: 1.
9. HARVEY, A. M., LILLIENTHAL, J. L., JR., AND TALBOT, S. A.: On the effects of the intra-arterial injection of acetylcholine and prostigmine in normal man. Bull. Johns Hopkins Hosp., 1941, 69: 529.
10. KOELLE, G. B.: Protection of cholinesterase against irreversible inactivation by di-isopropyl fluorophosphate in vitro. J. Pharmacol., 1946, 88: 232.
11. KOSTER, R.: Synergisms and antagonisms between physostigmine and di-isopropyl fluorophosphate in cats. J. Pharmacol., 1946, 88: 39.
12. RIKER, W. F., JR., AND WESCOE, W. C.: The direct action of prostigmine on skeletal muscle; its relationship to the choline esters. J. Pharmacol., 1946, 88: 58.

## BOOKS RECEIVED FOR REVIEW

- Actions of Radiations on Living Cells.* By D. E. LEA. Illus. 402 pp. \$4.50. The Macmillan Company, New York, New York, 1947.
- Bacteriology: Laboratory Directions for Pharmacy Students.* Compiled by MILAN NOVAK AND ESTHER MEYER. 2nd ed. 247 pp. \$2.75. The C. V. Mosby Company, St. Louis, Missouri, 1947.
- Brown-Sequard, Charles-Edouard. A Nineteenth Century Neurologist and Endocrinologist.* By J. M. D. OLMSTED. 253 pp. \$3.00. The Johns Hopkins Press, Baltimore, Maryland, 1946.
- Care of the Breast.* By ELSE K. LAROE. Illus. 240 pp. \$3.75. Froben Press, New York, New York, 1947.
- Color Atlas of Hematology.* By ROY R. KRACKE. Illus. 204 pp. \$5.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.
- Conduction Anesthesia.* By GEORGE P. PITKIN. Illus. 981 pp. \$18.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.
- Curare, Its History, Nature, and Clinical Use.* By A. R. MCINTYRE. Illus. 240 pp. \$5.00. The University of Chicago Press, Chicago, Illinois, 1947.
- Die Hormonalen Aspekte des Fortpflanzungsprozesses.* By DR. JULES SAMUELS. 152 pp. Holdert & Company, N.V., Amsterdam, Holland, 1946.
- Die Hormonversorgung des Foetus.* By DR. JULES SAMUELS. Illus. 320 pp. Leiden, Holland, 1947.
- Diseases of the Chest.* By ELI H. RUBIN. Illus. 685 pp. \$12.00. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.
- Diseases of Metabolism.* Edited by GARFIELD G. DUNCAN. 2nd ed. Illus. 1045 pp. \$12.00. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.
- Diseases of the Nose and Throat.* By CHARLES J. IMPERATORI AND HERMAN J. BURMAN. 3rd ed. Illus. 576 pp. \$12.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.
- Dolores Mortales. Algas Esplacnicas.* By DR. MIGUEL LOPEZ ESNAURRIZAR. 67 pp. Mexico, 1947.
- Dynamic Aspects of Biochemistry.* By ERNEST BALDWIN. 457 pp. Cambridge University Press, Cambridge, Great Britain, 1947.
- Essentials of Endocrinology.* By ARTHUR GROLLMAN. 2nd ed. Illus. 644 pp. \$10.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.
- Expectant Motherhood.* By NICHOLSON J. EASTMAN. 2nd ed. 198 pp. \$1.50. Little, Brown and Company, Boston, Massachusetts, 1947.
- Experiences with Folic Acid.* By TOM D. SPIES. Illus. 110 pp. \$3.75. The Year Book Publishers, Inc., Chicago, Illinois, 1947.
- Gynecology, including Female Urology.* LAWRENCE R. WHARTON. 2nd ed. Illus. 1027 pp. \$10.00. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.
- Handbook of Commonly Used Drugs.* By MICHEL PIJOAN AND CLARK H. YAEGER. 198 pp. \$3.75. Charles C. Thomas, Springfield, Illinois, 1947.

- If You Need an Operation.* DR. RICHARD A. LEONARDO. 198 pp. \$3.00. Froben Press, New York, New York, 1947.
- Lung, The.* By WILLIAM SNOW MILLER. 2nd ed. Illus. 222 pp. \$7.50. Charles C. Thomas, Springfield, Illinois, 1947.
- Method of Vitamin Assay.* Edited by THE ASSOCIATION OF VITAMIN CHEMISTS, INC. 189 pp. \$3.50. Interscience Publishers, Inc., New York, New York, 1947.
- Modern Dermatology and Syphilology.* By S. WILLIAM BECKER AND MAXIMILIAN E. OBERMAYER. 2nd ed. Illus. 1017 pp. \$18.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.
- Nurse-Patient Relationships in Psychiatry.* By HELENA WILLIS RENDER. Illus. 346 pp. \$3.00. The McGraw-Hill Book Company, New York, New York, 1947.
- Penicillin in Syphilis.* By JOSEPH EARLE MOORE. Illus. 319 pp. \$5.00. Charles C. Thomas, Springfield, Illinois, 1946.
- Penicillin Therapy.* By JOHN A. KOLMER. 2nd ed. Illus. 339 pp. \$6.00. D. Appleton-Century Company, New York, New York, 1947.
- Physical Medicine in General Practice.* Edited by ARTHUR L. WATKINS. 341 pp. \$5.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.
- Practical Physiological Chemistry.* By PHILIP B. HAWK, BERNARD L. OSER, AND WILLIAM H. SUMMERSON. 12th ed. Illus. 1323 pp. The Blakiston Company, Philadelphia, Pennsylvania, 1947.
- Rehabilitation Through Better Nutrition.* By TOM D. SPIES. Illus. 94 pp. \$4.00. 50 Fig. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.
- Retinal Structure and Colour Vision.* By E. N. WILLMER. Illus. 431 pp. \$4.50. The Macmillan Company, New York, New York, 1947.
- Surgeon's Domain, A.* By BERTRAM M. BERNHEIM. 253 pp. \$3.00. W. W. Norton & Company, Inc., New York, New York, 1947.
- Synopsis of Operative Surgery.* By H. E. MOBLEY. 2nd ed. Illus. 416 pp. \$6.00. The C. V. Mosby Company, St. Louis, Missouri, 1947.
- Techniques and Procedures of Anesthesia.* By JOHN ADRIANI. 404 pp. \$6.00. Charles C. Thomas, Springfield, Illinois, 1947.
- Textbook of Medicine.* Edited by RUSSELL L. CECIL. 7th ed. Illus. 1730 pp. \$10.00. W. B. Saunders Company, 1947.
- Tuberculosis as it Comes and Goes.* By EDWARD W. HAYES. 2nd ed. Illus. 220 pp. \$3.75. Charles C. Thomas, Springfield, Illinois, 1947.
- Uterotubal Insufflation.* By I. C. RUBIN. Illus. 453 pp. \$10.00. The C. V. Mosby Company, St. Louis, Missouri, 1947.
- Vascular Disorders of the Limbs.* By SIR THOMAS LEWIS. 2nd ed. 118 pp. \$2.25. The Macmillan Company, New York, New York, 1946.

# VIVAX RELAPSE RATES FOLLOWING CONTINUED ATABRINE SUPPRESSIVE MEDICATION: OBSERVATIONS ON MALARIA IN AN INFANTRY REGIMENT\*†

BENJAMIN M. BAKER,<sup>1</sup> AND DAVID PLATT,<sup>1,2</sup>

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## I. INTRODUCTION

A. The 147th Infantry Regiment served on various islands of the Pacific from the first half of 1942 to September, 1945. The malaria experience of the regiment, consisting of exposures to malaria of varying severity, atabrinization and deatabrinization, carefully recorded by different observers (1) (2), provides a basis for the study of several important problems concerning the control of the relapsing characteristic of vivax malaria. A period of heavy exposure was followed first by six months withdrawal of suppressive medication, then by eighteen months of well-controlled suppressive atabrinization, with little or no fresh exposure, and finally by terminal deatabrinization.

Although the data are neither complete nor perfect from a scientific standpoint, nevertheless opportunities for comparable studies are

\* Presented in slightly altered form before the meeting of the American Clinical and Climatological Association at Hershey, Pennsylvania, in October, 1946.

† Released for publication by the War Department, Office of the Surgeon General.

<sup>1</sup> Former Colonel, M.C., AUS, and Chief Consultant in Medicine, United States Army Forces, Pacific.

<sup>2</sup> Former Captain, M.C., AUS, and Surgeon, 147th Infantry Regiment.

<sup>3</sup> This study depended upon a carefully planned series of sequential observations and the participation both directly and indirectly of many individuals too numerous to mention by name. The initial demalarialization plan was formulated by the joint Army and Navy South Pacific Malaria and Epidemic Control Organization, first under the leadership of Commander James J. Sapero, M.C., USN, later Commander Fred F. Butler, M.C. USN, and Lt. Col. Paul A. Harper, M.C., AUS, the representative of the United States Army Forces. Lt. Commander W. G. Reddick, M.C., USNR, managed the demalarialization in Samoa, and Lt. Col. Wilbur G. Downs, M.C. AUS, made a special analysis of the Samoa results. The regimental surgeons provided the continuity upon which depended an indispensable record of individual malaria attacks.

likely to be infrequent in the future. Therefore, the information provided by this study on the practical management of relapsing malaria in heavily seeded troops merits record and publication.

B. *Facts relevant to the subject.* 1. Atabrine (0.1 gms./day) will suppress the vast majority, if not all, primary or recurrent attacks of vivax malaria (3), but thus far has not been shown to cure the disease in the same way that most, if not all, cases of falciparum malaria are cured by a like regime (4).

2. An outstanding and *specific* characteristic of vivax malaria is its tendency to relapse (5). The frequency and intensity of relapse vary with the *strain* of *P. vivax* (6) and, undoubtedly, also with factors peculiar to the host (7).

3. The tendency of vivax malaria to relapse does not continue indefinitely, but ceases spontaneously after two, three or possibly more years in all strains upon which there is reliable information (8).

C. *Significant gaps in knowledge relevant to the subject.* 1. From the facts cited in paragraph B, it seems probable that the majority of the normal relapses from vivax malaria would be prevented over a period of two or three years provided suppressive atabrine were given continuously. For obvious reasons, this theoretic possibility has never been checked under satisfactorily controlled experimental conditions; therefore, the proposition has never been proved.

2. Assuming the proposition to be true, the academic, but nonetheless scientifically important, questions arise as to whether the latent vivax infections were merely suppressed temporarily or were extinguished as a result of a) the medication or b) the sheer passage of time and the development of biologic defense.

## II. MATERIALS AND METHODS OF OBSERVATIONS

A. *Composition.* The regiment, a National Guard organization, was recruited largely from Ohio and contained no negroes. Training took place in the southern and southeastern parts of the United States in areas where on the whole malaria control was excellent. Although no information upon the amount of malaria acquired during training is available, it is altogether likely that it was negligible in amount and suppressive medication was not used until the regiment arrived in Guadalcanal.

B. *Pacific history.* The 147th Infantry Regiment arrived overseas

in two echelons, the first in April 1942 at Tonga, and the second in June 1942 at Fiji, neither of which islands is malarious. The First Battalion arrived at Guadalcanal, at the time a very malarious island, on 4 November 1942. Quinine sulphate, 0.3 gms. daily, was used as a malaria suppressive. The Third Battalion, plus special units, arrived 29 November 1942 and from that date atabrine, 0.4 gms. weekly, was the standard suppressive drug for all the regimental troops. The Second Battalion did not reach Guadalcanal until 7

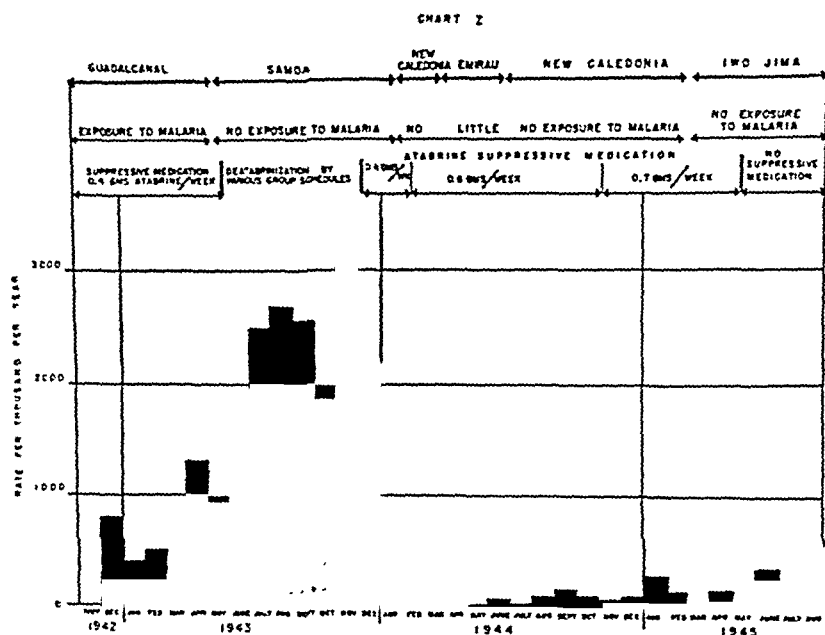


CHART I. MALARIA RATES, MALARIA EXPOSURE; ATABRINIZATION AND DEATABRINIZATION OF AN INFANTRY REGIMENT

February 1943. A few replacements previously unexposed to malaria joined the regiment during the middle of April. On 12 May 1943 the entire regiment left for British Samoa, a non-malarious island.

Thus the regimental components were exposed to hyperendemic malaria for periods varying from one to six months. During this exposure atabrine discipline was poor and even the introduction on 29 March 1943 of the roster system of administering the drug did nothing to lessen a high malaria rate (Chart I).



On Samoa, troops were released from atabrine suppressive medication. This project and its results are discussed in other reports and will be published in detail elsewhere by these several observers concerned, but may be briefly summarized as follows. One group stopped atabrine suddenly. A second took atabrine 0.1 gm. t.i.d. for seven days, rested for ten days, took similar doses of atabrine for the next seven days, and then discontinued medication. A third group stopped atabrine completely for ten days and was then treated according to the schedule for the second group. The fourth group took atabrine 0.1 gm. t.i.d. for seven days, rested for two days, took plasmochin 0.01 gm. b.i.d. for five days, rested for ten days, then repeated the entire cycle of atabrine and plasmochin therapy before stopping all medication. The fifth group continued the suppressive atabrine schedule (0.4 gms. weekly) for six weeks and then discontinued the drug. All clinical attacks were treated as follows:

1. Quinine sulfate or hydrochloride 0.6 gm. t.i.d. with atabrine 0.1 t.i.d. for three days.
2. Atabrine 0.1 gm. t.i.d. for an additional four days.
3. Rest two days.
4. Plasmochin 0.01 gms. b.i.d. for five days. Patients were given no further antimalarial drugs unless other clinical attacks developed, which were then treated in the same manner.

Over ninety per cent of the men with long Guadalcanal exposure had at least one attack of malaria on Samoa; about eighty-five per cent relapsed at least once. The highest malaria rate developed in the first group the third week after stopping suppression, and exceeded fourteen thousand per thousand per year. The peak of the first observed relapses was reached the sixth week following the first clinical attacks. This sustained high level of clinical reactivation makes it clearly evident that the men in the 147th Infantry Regiment had been infected with strains of *P. vivax* of a highly relapsing character.

Since its working efficiency was so severely depleted, the entire regiment returned to supervised atabrine suppressive medication, 0.4 gm. per week on 26 November 1943. The malarial rate promptly declined on this dosage and late in January 1944 the amount of drug was increased to 0.6 gm. per week.

The regiment left Samoa on 3 February 1944 for New Caledonia, a nonmalarious area, and exactly two months later departed for the Island of Emirau. This island had a history of moderate incidence of malaria but it is not believed that high levels of transmission prevailed during the period—less than three months—that the 147th remained there (Table II). An extensive program of drainage and larviciding virtually eliminated anopheles breeding. Natives were removed to another island or were confined in a well-controlled area. Such transmission as may have occurred must have been due to the residual population of anopheline adults present when the troops arrived. Atabrine discipline was excellent.

Early in July the regiment left Emirau and returned to non-malarious New Caledonia, remaining for eight months, after which it departed for Iwo Jima, arriving there on 20 March 1945. This island is also free from malaria. Throughout all these movements the regimental troops continued to take suppressive atabrine, the only variation in the program being an increase commencing in November 1944 in the weekly dosage from 0.6 to 0.7 gms. per week. Once established on Iwo Jima, consideration was given once more to the desirability of deatabrinizing the remaining troops, and on 20 May 1945 the suppressive drug program was officially discontinued.

In summary, therefore, the 147th Infantry Regiment, following its malaria exposure on Guadalcanal and its various deatabrinization experiences on Samoa, took suppressive atabrine from 26 November 1943 (Samoa) to 20 May 1945 (Iwo Jima), a period of eighteen months.

### III. OBSERVATIONS

This paper is concerned primarily with the relapse experience, with vivax malaria,<sup>4</sup> of the 147th Infantry Regiment after stopping atabrine suppressive medication. Observations were restricted to men who had remained with the organization from the beginning of their various group exposures on Guadalcanal until June, July or August, 1945. By September 1945 a considerable number of men had already been returned to the United States and those remaining were being returned so rapidly that the observations were discontinued.

Not all of these men had equal periods of malaria exposure on

<sup>4</sup> All diagnoses were confirmed by the demonstration of parasites in blood films.

TABLE I  
*Monthly malaria attack rates per thousand per year by groups,  
 November '42 through August '45*

	GROUP A		GROUP B		GROUP C		TOTAL	
	six months*		three months*		one month*		Strength	Rates
	Strength†	Rate	Strength†	Rate	Strength†	Rate		
Nov. 42	89	0					0	0
Dec. 42	89	809					89	809
Jan. 43	89	405					89	405
Feb. 43	89	1215	332	325			421	513
Mar. 43	89	809	332	72			421	228
Apr. 43	89	1484	332	1300	23	522	444	1296
May. 43	89	1348	332	794	23	1043	444	918
Jun. 43	89	2022	332	1913	23	522	444	1863
Jul. 43	89	4315	332	1986	23	2609	444	2484
Aug. 43	89	5527	332	1986	23	1565	444	2673
Sep. 43	89	4448	332	2222	23	2609	444	2538
Oct. 43	89	3774	332	1336	23	1565	444	1836
Nov. 43	89	4315	332	2491	23	2609	444	3132
Dec. 43	89	405	332	2744	23	522	444	2160
Jan. 44	89	270	332	505	23	0	444	432
Feb. 44	89	270	332	253	23	0	444	243
Mar. 44	89	135	332	108	23	0	444	108
Apr. 44	89	0	332	72	23	0	444	54
May 44	89	0	332	0	23	522	444	27
Jun. 44	89	135	332	72	23	0	444	81
Jul. 44	89	135	332	0	23	0	444	27
Aug. 44	89	0	332	144	23	0	444	108
Sep. 44	89	405	332	108	23	0	444	162
Oct. 44	89	270	332	72	23	0	444	108
Nov. 44	89	135	332	72	23	0	444	81
Dec. 44	89	135	332	108	23	0	444	108
Jan. 45	89	270	332	217	23	1043	444	270
Feb. 45	89	135	332	108	23	522	444	135
Mar. 45	89	270	332	0	23	0	444	54
Apr. 45	89	270	332	108	23	0	444	135
May 45	89	0	332	108	23	0	444	81
Jun. 45	85	706	324	222	23	522	432	333
Jul. 45	81	444	273	220	23	0	377	255
Aug. 45	50	1200	218	330	15	800	283	509

\* Time spent on Guadalcanal.

† Number of men remaining with regiment since arriving on Guadalcanal.

Guadalcanal. The differences are shown in Table I by groups, together with the respective relapse experiences of the groups.

It has been pointed out that the malaria exposure of the regiment on Emirau was not great. Table II gives the malaria rate of men who joined the regiment after it left Guadalcanal and provides strong evidence that malarialization of the regiment on Emirau was of little importance.

In brief, it is evident from the chart and the tables that the cessation of atabrine on 20 May 1945 was unattended by anything like the

TABLE II

*Monthly malaria attack rates per thousand per year of 147th infantry men with Emirau but not Guadalcanal malaria exposure*

## Group D

	STRENGTH	RATE
Apr. 44	803	0
May 44	803	15
Jun. 44	803	45
Jul. 44	803	0
Aug. 44	803	45
Sep. 44	803	0
Oct. 44	803	0
Nov. 44	803	30
Dec. 44	803	0
Jan. 45	803	15
Feb. 45	803	15
Mar. 45	803	15
Apr. 45	803	15
May 45	803	0
Jun. 45	789	15
Jul. 45	767	16
Aug. 45	745	16

increase in attack rates observed following trial deatabrinization in Samoa almost exactly two years before. Very slight rate excesses in June, July and August over those of previous months are apparent but their significance is not known. It is unfortunate that observations could not have been continued longer to determine this point.

## IV. DISCUSSION

As indicated above, vivax malaria relapses over a period of many months, the great bulk of clinical reactivity occurring within a year after the initial attack but extending over into the second and occasionally even the third years. This information is derived from

observations on both mosquito-induced therapeutic malaria and cases of naturally acquired malaria removed from the possibility of reinfection (5) (7) (9) (10). The actual frequency of reactivations after initial episodes is well known for the strain or strains of vivax malaria under discussion (2) (11), but it is well established, too, that different strains of vivax vary in respect to their characteristic relapse behavior (6).

It seems logical to infer on the basis of available information that a high degree of clinical activity would have manifested itself for at least a year, in some cases even longer, following the last attacks acquired on Guadalcanal, i.e., until June or July 1944. This inference is supported by the relapse experience of the 147th Infantry Regiment from June to November 1943, and also by the study of several groups of men who acquired malaria on Guadalcanal and when subsequently removed to non-malarious areas continued to exhibit reactivation for well over a year. Therefore, it is highly probable that the reduction in the number of relapses in this regiment after December 1943 was due to the resumption of suppressive atabrine and not to a spontaneous termination of clinical activity. This conclusion is justified even though we can not demonstrate what the curve of attack rates from January 1943 to May 1945 would have been in the absence of drug suppression. This curve could have been determined only by denying atabrine to a representative segment of the regiment, an experiment which under the circumstances was not feasible. Such a procedure, could it have been carried out, would have made a controlled experiment out of what must remain an uncontrolled, though informative, series of observations.

The continuation of suppressive atabrine medication in troops as heavily seeded as this regiment was a military necessity, for without it high malaria rates and serious disability would have prevailed. When necessity demanded that this plan be followed, serious consideration was given to the ultimate fate of such troops, but there were no data upon which to base reliable predictions. It was realized and feared that prolonged atabrine suppression might merely postpone the evil day, as was known to be the case when short term suppressive treatment was employed. Provided that a three months' period of

observation following final deatabrinization of heavily seeded troops is sufficient, the observations recorded here answer a very important question.<sup>5</sup> Not only is the high malaria rate of heavily seeded troops temporarily controlled by atabrine suppressive medication but a highly significant amount of clinical vivax malaria is permanently abolished.

#### V. CONCLUSIONS

1. It has been shown that standard suppressive atabrine treatment may be discontinued safely two years after the last exposure of heavily seeded troops without leading, within a period of three months, to an excessive reactivation rate.

2. Previous observations of the same troops proved that it was not safe to *stop* atabrine for at least six months after exposure.

3. It is generally accepted that four weeks of suppressive atabrine medication following the acquisition of *P. falciparum* infection prevents all further clinical activity. It appears from these observations that suppressive atabrine medication for eighteen months after significant exposure exerts a comparable effect upon *some* but *not all* cases of *P. vivax* infection.

4. This illustrates and confirms the general belief that heavily seeded troops should be taken off suppressive medication very cautiously. If the relapse characteristics of the strains involved resemble those of Guadalcanal strains, troops should not be taken off for at least six months. From then on suppressive medication should be withdrawn only from sample fractions to obtain some indication of the remaining relapse latencies. After two years it is probably safe to discontinue the suppressive drug program for all troops at the same time.

5. These observations are believed to indicate that, had it been possible to make troops ingest atabrine in suppressive doses faithfully and for a sufficiently long time after malaria exposure, clinical malaria in the armed forces might have been in large measure prevented.

<sup>5</sup> All men who participated in this study were requested to report their subsequent attacks of malaria to the senior author by letter. Up to January, 1947, only five attacks were reported.

## REFERENCES

- (1) REDDICK, W. G., Lt. Commander, M.C. USNR.: Special Report to Officer-in-Charge Malaria Control, South Pacific Area and South Pacific Force (Nov.) 1943.
- (2) DOWNS, W. G., Lt. Col. M.C. AUS.: Results in an Infantry Regiment of Several Plans of Treatment for Vivax Malaria. In Press.
- (3) SCHAFER, ALEXANDER J., Lt. Col. M.C. AUS., LEWIS, ROGER A., Capt. M.C. AUS, AND BAKER, BENJAMIN M., Col. M.C. AUS.: The Suppression of Malaria, Special Report to the Surgeon General, United States Army, (July) 1944.
- (4) Malaria Report #191, Board for the Coordination of Malaria Studies, Summary of Research on Malaria Conducted in the Cairns Area by the Director of Medicine R.A.M.C., Brig. N. H. FAIRLEY, (Aug.) 1944.
- (5) BOYD, M. F.: The Infection in the Intermediate Host. Symptomatology, General Consideration in a Symposium on Human Malaria, Pub. 15, Amer. Assoc. for the Advancement of Science, Washington, D. C., 1941.
- (6) BOYD, M. F. AND KITCHEN, S. F.: Recurring Clinical Activity in Infections with the McCoy Strain of Plasmodium Vivax. Amer. J. Trop. Med. 17: 833, 1937.
- (7) HACKETT, L. W.: Malaria in Europe. Oxford University Press, London. Humphrey Milford, 1937.
- (8) BOYD, MARK F.: Present Day Problems of Malaria Infection. J. A. M. A. 124: 1179, (April) 1944.
- (9) BOYD, MARK F. AND KITCHEN, S. F.: Renewed Clinical Activity in Naturally Induced Vivax Malaria. Amer. J. Trop. Med. 24: 4; (July) 1944.
- (10) Malaria Report #337, Board for the Coordination of Malaria Studies, Interval Report on the Use of SN 6911 for the Treatment of Vivax Malaria at the Harmon General Hospital through the Medical Division, Office of the Surgeon General, United States Army.
- (11) SHAFER, ALEXANDER J., Lt. Col. M.C. AUS., LEWIS, ROGER A., Capt. M.C. AUS, AND BAKER BENJAMIN M., Col. M.C. AUS: Malaria in the 164th Infantry Regiment. Special Report to the Surgeon General, United States Army, 1943.

# THE INFLUENCE OF HYPERVITAMINOSIS A ON BONE GROWTH

THOMAS E. VAN METRE, JR.<sup>1</sup>

*From the Department of Pathology, Harvard Medical School, Boston, Mass.*

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The objective of this experiment was to determine the effect of hypervitaminosis A on the longitudinal growth of the long bone of the rat. Collazo showed that large amounts of vitamin A will produce fractures in the long bones of growing rats (1) (2) (3). This work has been duplicated by others. The literature on the subject has been adequately reviewed by Wolbach and Bessey (4) and by T. Moore and Wong (5). Wolbach (6) has recently shown that the effect of hypervitaminosis A on the growing bone is a composite of several factors: first, a speeding up of maturation, degeneration and death of cartilage cells in the epiphysis; second, correspondingly active replacement bone formation with early closure of epiphyses that normally close, and third, concurrent acceleration of remodelling of the shaft of the bone, normal in location and execution, but sufficiently abnormal in rate so that the rapid replacement of strong old bone with new weak bone produces a structure insufficiently strong, because of the normal lag in calcification, to resist fracture under the strains imposed by normal activity. Remodelling of the long bone has been interpreted as a function of stress and strain for two reasons—first, bone adapts itself in external form and internal trabeculation so that a maximum of strength is achieved with a high degree of economy of material; second, as Glucksman (8) (9) has well shown, bone in tissue culture grows in such a form that it most efficiently resists the strains applied to it. It therefore seemed desirable to Wolbach to correlate the accelerated remodelling of bone resulting from excessive vitamin A administration with the rate of longitudinal growth of bone, in order to ascertain whether remodelling is solely the result of mechanical factors. This experiment was therefore performed in his laboratory, under his direction, to ascertain whether or not an increased rate of longitudinal growth was present.

<sup>1</sup> Present address Johns Hopkins Hospital, Baltimore, Maryland.



## MATERIALS AND METHODS

The experiment consisted essentially of measuring longitudinal growth of the left tibia, body weight, and food intake of a number of rats given varying excessive doses of vitamin A.

The rats were albino rats of the Wistar strain, obtained from the Harvard Biological Laboratories. They were 21 day old "weanlings" at the onset of the experiment.

The vitamin A used was winterized, vitamin A concentrate, containing 500,000 international units per gram, distilled from fish liver and vegetable oils by Distillation Products, Inc. This compound contains vitamin A 14.3%, unidentified sterols less than 10%, tocopherols less than 1% in solution in distilled fat. The vitamin D content is well under 100 units per gram. (10) This preparation was administered orally, the given amount being placed on the back of the animal's tongue. The dosage of vitamin A, the number of animals treated, and the duration of the experiment is indicated in the graph summarizing the results. It was previously determined (4) (6) that crystalline vitamin A (obtained from the Distillation Products, Inc., Rochester, New York) produced all of the bone changes concerned in this experiment.

Bone growth was measured by taking weekly roentgenograms of the left tibia. To eliminate the necessity of using anesthesia, a special frame was designed for holding the animals motionless (Fig. 1). It was not difficult to secure the animals by tightening the tapes with the peg-winches and proper application of the spring clips. The distance on the roentgenograms between the midpoints of the proximal and distal ends of the bone was measured with calipers and considered to represent the length of the bone. With the x-ray tube set at a target-film distance of 90 cm. to insure perpendicular rays, minor variations in the position of the leg inherent in this technique did not introduce an error of more than 0.1 mm. Measurements with considerable comparative value, therefore, could be obtained.

Weight gain was followed by weighing the animals daily. Food intake was measured each day. Purina dog chow checkers were used as food. An amount somewhat above the daily expected intake was weighed out and put into the cages. At the end of twenty-four hours,

the intake was calculated after weighing the residue. The animals were housed in separate cages with wire mesh floors which allowed a certain loss of food, introducing an error into these measurements; however, being relatively constant throughout, this loss did not

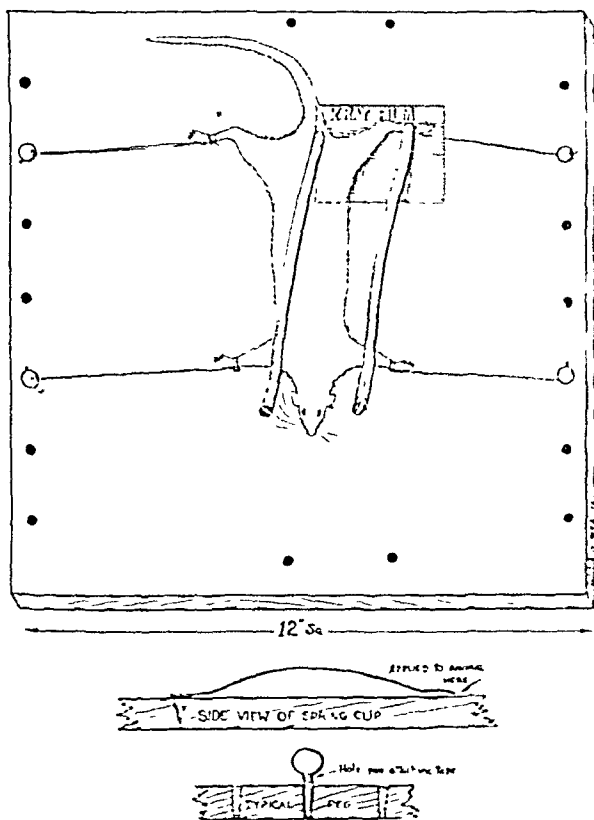


FIG. 1. APPARATUS FOR HOLDING ANIMALS WHILE ROENTGENGRAMS ARE BEING TAKEN

particularly distort the comparative value of the figures obtained. The animals were allowed free access to water.

Alterations in the gross morphology of the left tibia were followed by roentgenograms and observation of the bone at autopsy. Changes in histology were followed by study of microscopic sections.

## RESULTS

Weanling rats receiving 250, 275, 625, or 1250 international units of vitamin A per gram of body weight per day developed changes in the left tibia similar to those described by Wolbach (6).

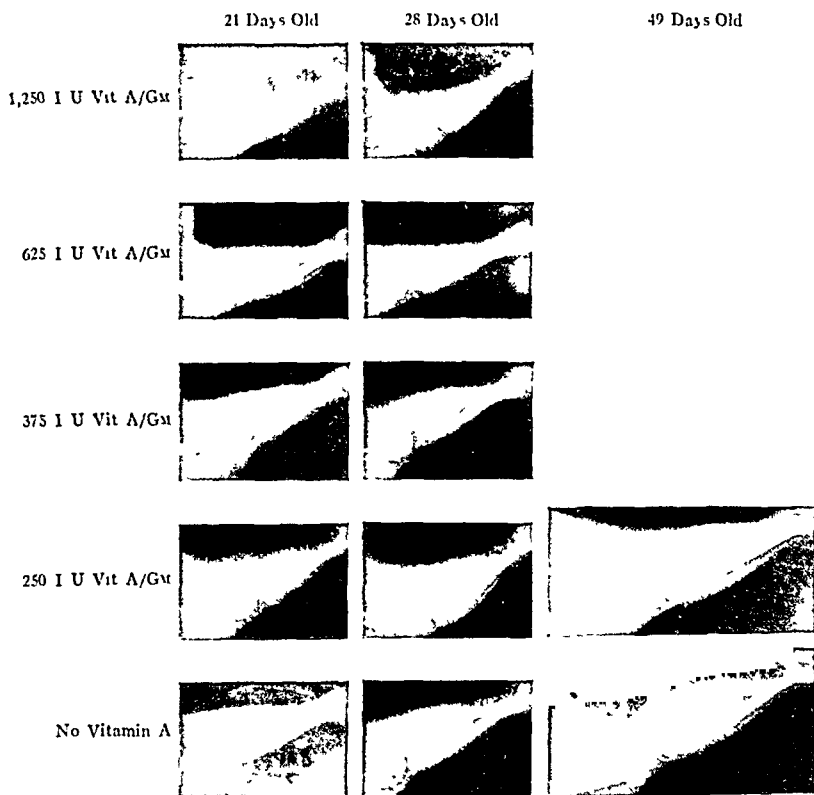


FIG. 2. The influence of hypervitaminosis A on the gross morphology of the left tibia of the growing rat as revealed by roentgenograms. The indicated amount of vitamin A was given every day beginning just after the "21 days old" X-ray was taken.

The gross changes are shown in the reproductions of the roentgenograms in Figure 2. The bones are seen to be more slender than normal controls. The cortex of the metaphysis is less opaque to the roentgen ray. The epiphyseal cartilage is decreased in width. Within the range of dosage used, the greater the amount of vitamin A given,

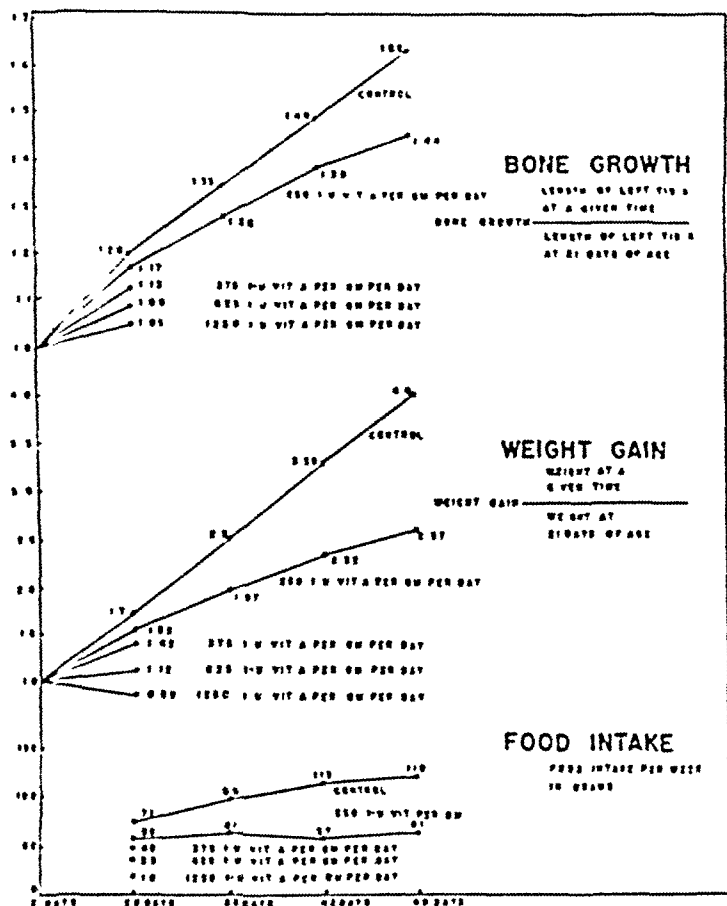


FIG. 3. THE INFLUENCE OF HYPERVITAMINOSIS A ON BONE GROWTH, WEIGHT GAIN AND FOOD INTAKE

Number of Animals

- Control: No Vit. A supplement: 16 rats for 1st week; 8 thereafter  
 250 1-U Vit. A per gm. per day: 12 rats for 1st week; 8 thereafter  
 375 1-U Vit. A per gm. per day: 10 rats  
 625 1-U Vit. A per gm. per day: 16 rats  
 1250 1-U Vit. A per gm. per day: 16 rats

the more pronounced the effect. These roentgenograms were taken too early to demonstrate fracture. Fracture was always observed, however, across the region of decreased density between the 7th and 14th day of treatment when 625 or 1250 international units of vitamin

A per gram of body weight per day were given, but only after the fourth week of treatment when 250 international units of vitamin A were given.

The microscopic changes were entirely similar to those described by Wolbach (6). Indeed, some of his observations were made on sections taken from these very animals. *The magnitude of change again was directly proportional to the amount of vitamin A administered.*

Coincidental to these gross and microscopic changes, and likewise increasing in degree in direct proportion to the amount of vitamin A given, was retardation of longitudinal growth of the left tibia, weight gain, and food intake as shown in Figure 3.

#### DISCUSSION

These animals were given a fish oil concentrate containing vitamin A. Under the experimental condition outlined, this distillate produced morphologic changes in the long bones of rats identical with those described by Wolbach (6); morphologic changes which he has produced using crystalline vitamin A as well as a similar distillate; morphologic changes which are therefore almost surely specific manifestations of hypervitaminosis A. It is felt in agreement with Wolbach and for the reasons which he advances that these morphologic changes may be interpreted as due to the acceleration of normal processes; to wit, the acceleration of maturation, degeneration, and death of epiphyseal cartilage cells, replacement bone formation, and the acceleration of normal remodelling of the bone. These morphologic changes may be produced by all of the dosages of vitamin A administered, but are the more pronounced the larger the dose of vitamin A given. Coincidental with these morphologic changes and again proportional to the amount of vitamin A given is a retardation of longitudinal bone growth. Therefore, in hypervitaminosis A, those bones that elongate most slowly because of larger doses, remodel most quickly. Accelerated longitudinal growth is therefore not the factor that makes these bones remodel more rapidly than normal bones. It seems likely, therefore, as Wolbach has already suggested (6), that some factor other than stress and strain must be important in the rapid remodelling seen in hypervitaminosis A.

These experiments establish the fact that in hypervitaminosis A longitudinal growth of long bones is retarded. They do not explain

why this phenomenon occurs. The reduction in food intake, which so closely parallels the reduction of bone growth and similarly is proportional to the amount of vitamin A given, may be one factor responsible. The proportional diminution in weight gain suggests that this retardation of growth may apply to tissues other than bone. The mechanism of growth retardation and the extent of its influence in hypervitaminosis A warrant further investigation.

#### CONCLUSIONS

Under the experimental conditions outlined, it has been demonstrated that:

(1) Hypervitaminosis A produced accelerated remodelling of the tibia of the growing "weanling" rat. This acceleration was proportional to the amount of vitamin A given.

(2) Hypervitaminosis A in the same animals produced retardation of longitudinal growth of the tibia. This retardation was proportional to the amount of vitamin A given.

It is, therefore, possible to have accelerated remodelling of bone that is secondary to no readily appreciable increase in the stress and strain operating in and on that bone.

# EXPERIMENTAL ANAPHYLACTIC LESIONS OF THE CORONARY ARTERIES OF THE "SCLEROTIC" TYPE, COMMONLY ASSOCIATED WITH RHEUMATIC FEVER AND DISSEMINATED LUPUS ERYTHEMATOSUS

ARNOLD R. RICH AND JOHN E. GREGORY<sup>1</sup>

*The Department of Pathology, The Johns Hopkins University School of Medicine*

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It has been recognized for many years that narrowing of the branches of the coronary arteries as a result of intimal proliferation occurs commonly in rheumatic fever. This lesion, in its more advanced and fibrous form, often closely resembles and, indeed, may become indistinguishable from ordinary arteriosclerosis. In earlier stages, however, the lesions are usually distinguishable from ordinary arteriosclerosis by their inflammatory component. The inflammation may be confined to the intima, or it may involve the entire vessel wall. Focal necrosis of the media is frequent, and well-marked early lesions of this type have the necrotizing-inflammatory characteristics of periarteritis nodosa. This was pointed out years ago by Aschoff (1) in his original paper on rheumatic carditis, and has been confirmed repeatedly. The healing of such lesions may leave only a thickened, fibrous intima, or there also may be focal scars in the media if medial necrosis has occurred. These various lesions of the coronary arteries, which occur with great frequency in rheumatic fever, have been well described by Perry (2), Klinge (3), Karsner and Bayless (4), Coombs (5), Gross, Kugel and Epstein (6), and others, and they are familiar to all students of the pathology of rheumatic fever.

It is well known that intimal proliferation occurs in periarteritis nodosa, and that in older lesions the thickened intima becomes fibrous, permanently narrowing the lumen of the vessel. That periarteritis nodosa in man can result from protracted hypersensitive reactions of the anaphylactic type has been shown by one of us (8) (9) (10), and we have demonstrated that this vascular lesion can be produced experi-

<sup>1</sup> At present Captain in U. S. Army Medical Corps, Walter Reed General Hospital, Washington, D. C.

mentally by subjecting animals to anaphylactic reactions of the serum sickness type (11). In the report of those experiments, we drew attention to the fact that the lumen of some of the affected arteries became narrowed as a result of intimal proliferation. We have also shown experimentally that valvular, endocardial and myocardial lesions with the basic characteristics of rheumatic carditis can be caused by protracted hypersensitive reactions of the anaphylactic type (12). Hopps and Wissler (13), Hawn and Janeway (17), and Crockett and Roberts (7) have confirmed these experimental results. In other studies we have pointed out that alveolar capillary damage and thrombosis, of the type that characterizes rheumatic pneumonitis, can result in man from anaphylactic hypersensitive reactions to drugs (14), and can occur during protracted anaphylactic reactions in experimental animals (15). We have presented still other forms of evidence in support of the view that the lesions of rheumatic fever, in whatever tissue they are found, may represent effects of an antigen-antibody reaction such as that which occurs in protracted hypersensitive reactions of the anaphylactic type (12) (18). The present observations add to the previously accumulated evidence the fact that the familiar "rheumatic coronary sclerosis" can, likewise, result from protracted anaphylactic reactions to foreign antigens.

This study comprises 45 rabbits which were sensitized to horse serum in a manner that is known to be favorable to the production of the serum sickness type of protracted anaphylactic reaction both in man and in the rabbit, namely, the intravenous injection of large amounts of the antigen. Male albino rabbits, averaging about 2 kg. in weight were used. Sterile horse serum without preservative<sup>2</sup> was injected into the ear vein in a dosage of 10 cc. to 15 cc. per kilogram. Seven of the forty-five animals received only a single injection; twenty-five received two injections, and thirteen received five to ten injections at various intervals. The animals were killed at periods between 17 and 364 days following the first injection of serum, and the coronary arteries were carefully studied microscopically in appropriate sections of the heart. The vessels in most of the other viscera were similarly studied.

<sup>2</sup> The serum was kindly provided by the Medical-Research Division of Sharp and Dohme, Inc.



All of the forty five animals became skin sensitive. Eight responded to the intracutaneous injection of 0.1 cc. of horse serum on the flank, with a severe Arthus phenomenon, i.e., edema, erythema, haemorrhage, necrosis and ulceration; fourteen with edema and haemorrhage; nineteen with an area of edema and erythema four centimeters in diameter or larger; and four with an area of edema and erythema less than four centimeters in diameter. All of the animals in which lesions of the coronary arteries were found at autopsy had developed the serum sickness type of reaction (fever, erythema) following one or more of the intravenous injections of serum, but arterial lesions were not found in all animals which had exhibited this type of reaction.

Eighteen of the forty-five sensitized rabbits developed lesions of the coronary arteries of the type familiar in rheumatic fever. All stages and degrees of this vascular alteration were encountered. Figs. 1 through 13 illustrate the more advanced forms in comparison with examples of human "rheumatic coronary sclerosis." Three of the eighteen animals that developed the coronary lesions had received only one injection of horse serum; thirteen had received two injections; one had received five and one nine injections. All except two of the sensitized animals in which the vascular lesions were found were killed between nineteen and forty-six days after the first injection of serum.

We have not observed lesions of this type in the coronary arteries of non-sensitized rabbits used in this laboratory in other connections. During the course of these experiments a group of eighteen male albino rabbits, weighing approximately 2000 gms. each, were kept as controls in the same room under identical conditions of housing and diet. They received no serum. These controls were allowed to live from 86 to 134 days, i.e. they remained in the laboratory two to seven times as long as most of the sensitized animals that developed the vascular lesions. No lesion of the coronary arteries was found in any of these control animals.

Fibrotic intimal lesions, similar to those occurring in the coronary arteries, were found in peripheral arteries (thymus, epididymis, mesentery) of some of the sensitized animals. It may be noted that fibrotic narrowing of the lumen of small branches of the hepatic artery that are embedded in the scars of lesions produced by coccidia in the rabbit's

liver may be encountered in control animals, and bear no relation to sensitizing procedures. The "sclerosis" of a branch of the hepatic artery regarded by Sato (16) as a result of the injection of foreign protein appears to be a lesion resulting from coccidiosis, for the illustrated narrowed artery is embedded in a scar of the coccidiosis type.

One of us (18) has recently pointed out that disseminated lupus erythematosus exhibits, in common with rheumatic fever and periarteritis nodosa, an impressive variety of lesions, each of which occurs in protracted anaphylactic reactions of the serum sickness type, namely, fever, urticarial and erythematous cutaneous eruptions, purpura, arthritis, necrotizing-inflammatory arterial lesions, focal collagen degeneration, focal necroses of lymph nodes and of spleen, myocarditis, valvulitis, sterile inflammation of serous membranes, sterile pneumonitis, transient paresis. It is of no little interest, therefore, that in disseminated lupus erythematosus there occur "sclerotic" lesions of the coronary arteries of the same type as those that occur in rheumatic fever, in periarteritis nodosa and in our animals subjected to experimental serum sickness. That these lesions are related to disseminated lupus itself, and are not coincidental arteriosclerotic lesions, is evident from the fact that they occur even in children with the disease. Figures 14 and 15 illustrate lesions of this type in a child 11 years old, who had typical clinical and pathological disseminated lupus erythematosus.

#### SUMMARY

Lesions of the coronary arteries, altogether comparable with the "sclerosis" of the branches of the coronary arteries caused by rheumatic fever, occurred in 18 of 45 rabbits subjected to protracted anaphylactic reactions of the serum sickness type, and were not found in control rabbits. This observation adds further evidence to that which we have previously presented in support of the view that the various lesions of rheumatic fever can result from antigen-antibody reactions of the anaphylactic type.

In disseminated lupus erythematosus, also, coronary "sclerosis" of the same type occurs, and a wide variety of other lesions that are characteristic of protracted anaphylactic reactions.

## REFERENCES

1. ASCHOFF, L.: Zur Myokarditisfrage. *Verh. d. deutsch. path. Gesellschft.*, 1904, **8**: 46.
2. PERRY, C. B.: The main branches of the coronary arteries in acute rheumatic carditis. *Quart. Jour. Med.*, 1929-30, **23**: 241.
3. KLINGE, F.: Ker Rheumatismus. *Ergeb. d. allg. Path.*, 1933, **27**: 1.
4. KARSNER, H. T., AND BAYLESS, F.: Coronary arteries in rheumatic fever. *Am. Heart Jour.*, 1934, **9**: 557.
5. COOMBS, C. F.: Rheumatic Heart Disease. Wm. Woods and Co., N. Y., 1924.
6. GROSS, L., KUGEL, M. A., AND EPSTEIN, E. Z.: Lesions of the coronary arteries and their branches in rheumatic fever. *Am. Jour. Pathol.*, 1935, **11**: 253.
7. CROCKETT, K. A., AND ROBERTS, R. C.: Hypersensitivity Reactions. Presented at the 28th Annual Session of The American College of Physicians, Chicago, May 1, 1947.
8. RICH, A. R.: The rôle of hypersensitivity in periarteritis nodosa, as indicated by seven cases developing during serum sickness and sulfonamide therapy. *Bull. Johns Hopkins Hosp.*, 1942, **71**: 123.
9. RICH, A. R.: Additional evidence of the rôle of hypersensitivity in the etiology of periarteritis nodosa. *Bull. Johns Hopkins Hosp.*, 1942, **71**: 375.
10. RICH, A. R.: Hypersensitivity to iodine as a cause of periarteritis nodosa. *Bull. Johns Hopkins Hosp.*, 1945, **77**: 43.
11. RICH, A. R., AND GREGORY, J. E.: The experimental demonstration that periarteritis nodosa is a manifestation of hypersensitivity. *Bull. Johns Hopkins Hosp.*, 1943, **72**: 65.
12. RICH, A. R., AND GREGORY, J. E.: Experimental evidence that lesions with the basic characteristics of rheumatic carditis can result from anaphylactic hypersensitivity. *Bull. Johns Hopkins Hosp.*, 1943, **73**: 239.
13. HOPPS, H. C., AND WISSLER, R. W.: Experimental production of generalized arteritis and periarteritis (periarteritis nodosa). *Jour. Lab. and Clin. Med.*, 1946, **31**: 939.
14. RICH, A. R., AND GREGORY, J. E.: On the anaphylactic nature of rheumatic pneumonitis. *Bull. Johns Hopkins Hosp.*, 1943, **73**: 465.
15. GREGORY, J. E., AND RICH, A. R.: The experimental production of anaphylactic pulmonary lesions with the basic characteristics of rheumatic pneumonitis. *Bull. Johns Hopkins Hosp.*, 1946, **78**: 1.
16. SATO, Y.: Über die spezifischen Organveränderungen der Kaninchen bei wiederholter, parenteraler Eiweisszufuhr. *Trans. Soc. Pathol. Jap.*, 1934, **24**: 293.
17. HAWN, C. V., AND JANEWAY, C. A.: Histological and serological sequences in experimental hypersensitivity. *Jour. Exp. Med.*, 1947, **85**: 571.
18. RICH, A. R.: Hypersensitivity in disease, with especial reference to periarteritis nodosa, rheumatic fever, disseminated lupus erythematosus and rheumatoid arthritis. Harvey Lecture, 1946.

## EXPLANATION OF ILLUSTRATIONS

(Photomicrographs by Miss Marjorie Jackson)

FIG. 1. Branch of coronary artery from a case of rheumatic fever in a 5 year old child, with Aschoff bodies in myocardium (Autopsy 13, 551).

FIG. 2. Branch of coronary artery from rabbit (63), which had received 2 injections of horse serum, 10 cc./kg. each, and was killed 28 days after the first injection.

FIG. 3. Branch of coronary artery from a case of rheumatic fever in a male 24 years old, with Aschoff bodies in myocardium (Autopsy 18, 556).

FIG. 4. Branch of coronary artery from same case as Fig. 1.

FIG. 5. Branch of coronary artery from rabbit (64), which had received 2 injections of horse serum, 10 cc./kg. each, and was killed 28 days after the first injection.

FIG. 6. Branch of coronary artery from a case of rheumatic fever in a child 6½ years old, with Aschoff bodies in myocardium (Autopsy 7814).

FIG. 7. Branch of coronary artery from rabbit (83), which had received 9 injections of horse serum, 10 cc./kg. each, during 260 days.

FIG. 8. Branch of coronary artery from case of rheumatic fever in a youth 17 years old, with Aschoff bodies in myocardium (Autopsy 9181).

FIG. 9. Branch of coronary artery from rabbit (87), which had received 5 injections of horse serum, 10 cc./kg. each, during 122 days.

FIG. 10. Branch of coronary artery from rabbit (7), which had received 10 cc./kg. of horse serum and was killed 28 days later.

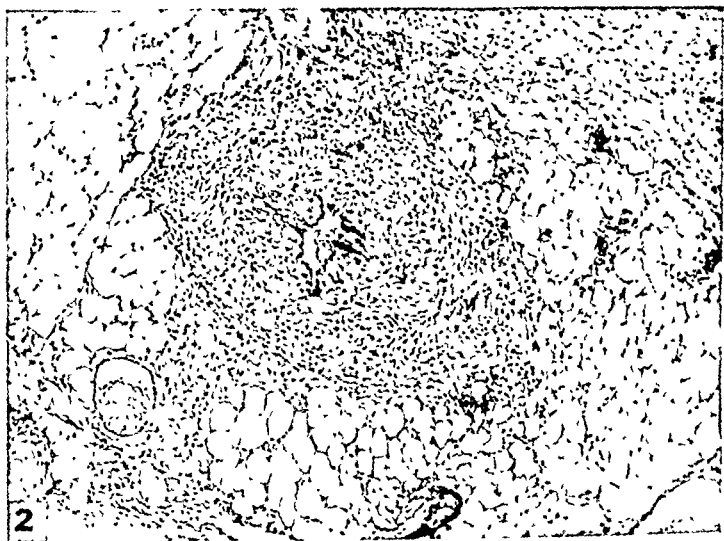
FIG. 11. Branch of coronary artery from rabbit (8), which had received 2 injections of horse serum, 10 cc./kg. each, and was killed 26 days after the first injection.

FIG. 12. Larger branch of coronary artery from same case of rheumatic fever as Fig. 8. The position of the internal elastic membrane is outlined with ink.

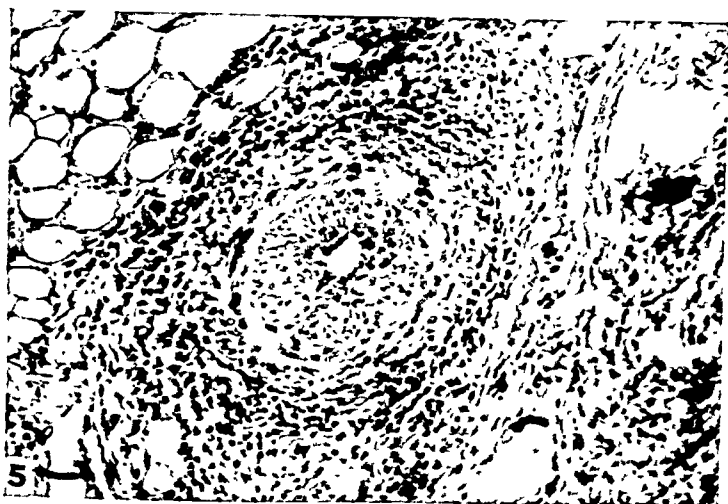
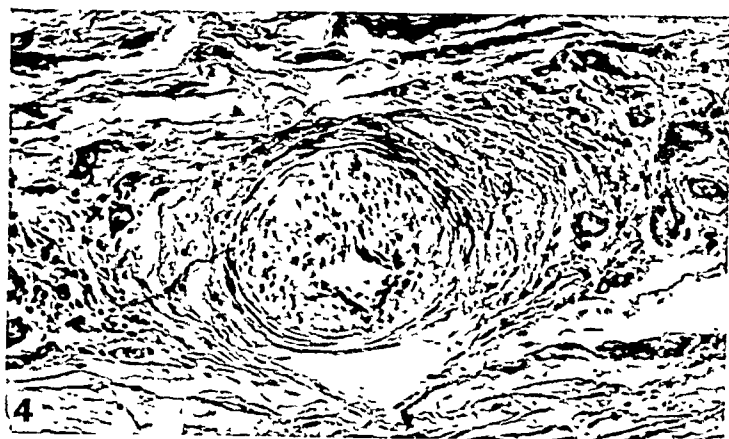
FIG. 13. Larger branch of coronary artery from rabbit (65), which had received 2 injections of horse serum, 10 cc./kg. each, and was killed 28 days after the first injection. The position of the internal elastic membrane is outlined with ink.

FIG. 14. Branch of coronary artery from a case of disseminated lupus erythematosus in a child 11 years old (Autopsy 20,354). Compare with Figs. 3, 4 and 5.

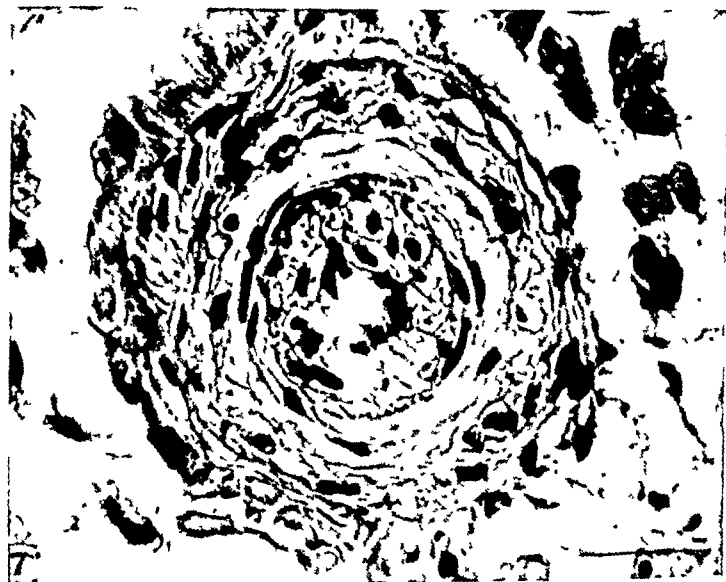
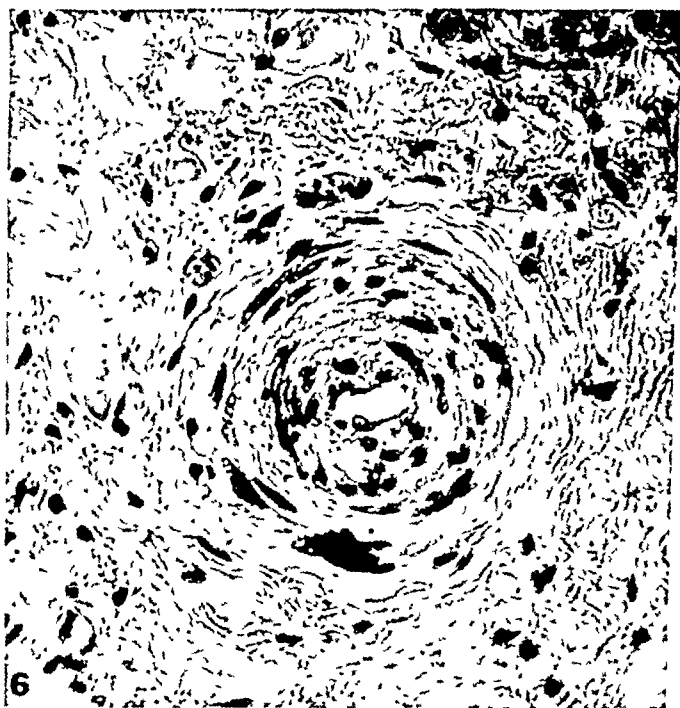
FIG. 15. Larger branch of coronary artery from same case of disseminated lupus erythematosus as in Fig. 14. The position of the internal elastic membrane is outlined with ink. Compare with Figs. 12 and 13.

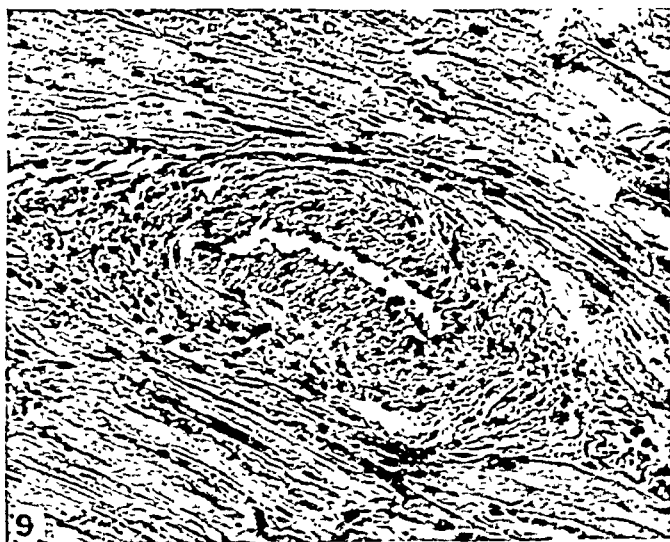
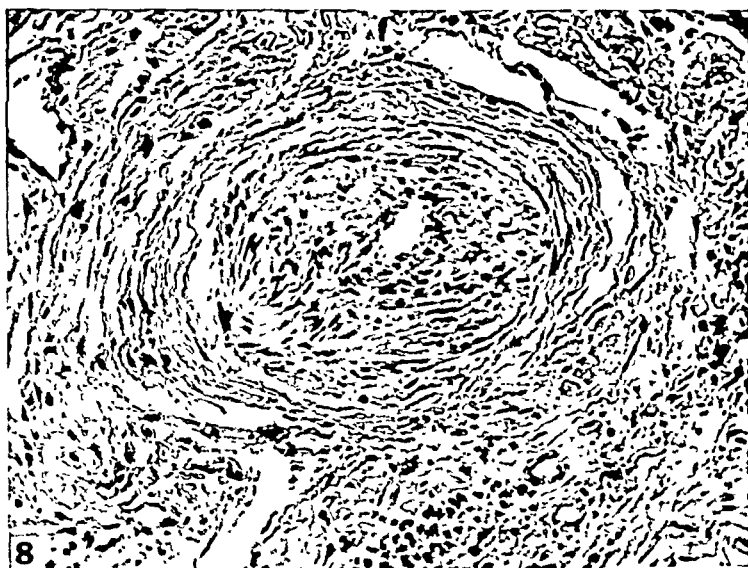


FIGS. 1 AND 2



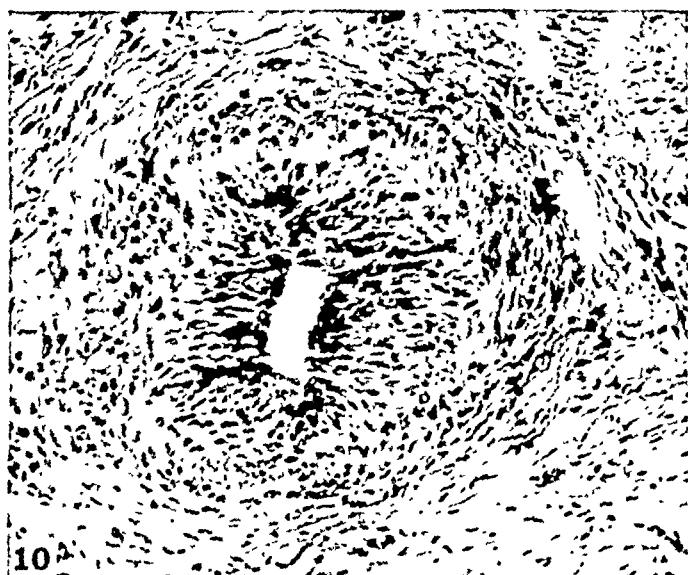
FIGS. 3, 4 AND 5



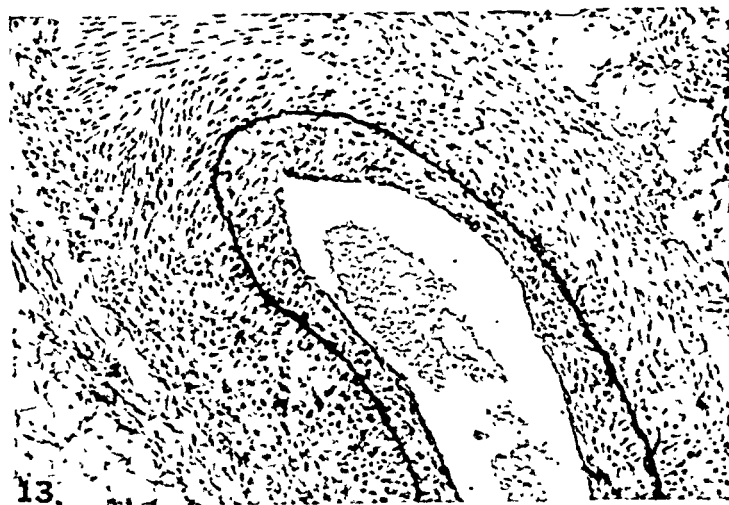
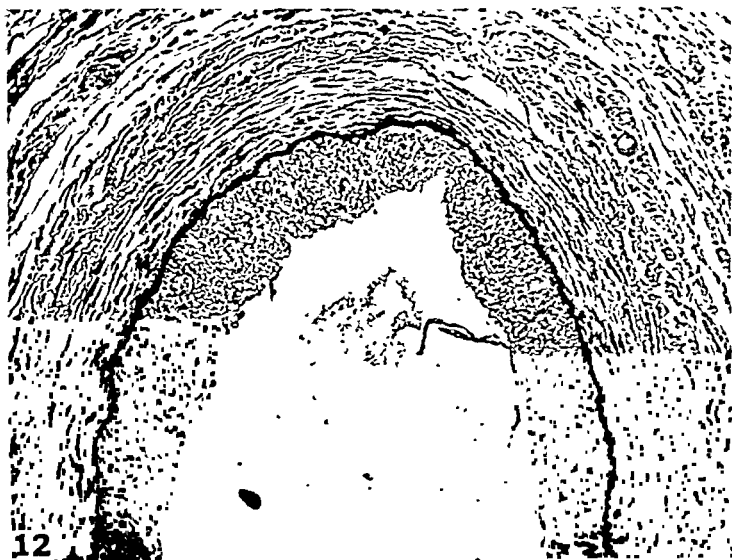


FIGS. 8 AND 9

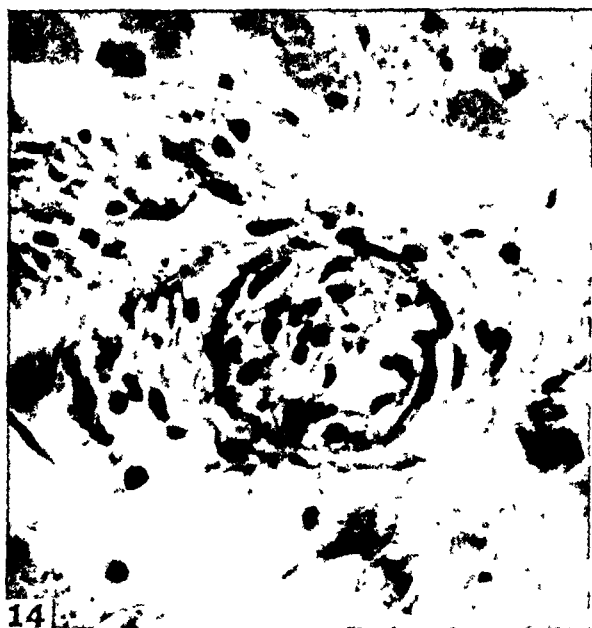




FIGS. 10 AND 11



FIGS. 12 AND 13



FIGS. 14 AND 15

# OBSERVATIONS ON AN EPIDEMIC OF INFLUENZA B OCCURRING AT THE JOHNS HOPKINS HOSPITAL IN NOVEMBER-DECEMBER, 1945<sup>1</sup>

THOMAS G. WARD AND GLEN R. LEYMASTER<sup>2</sup>

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During November and December, 1945, an outbreak of respiratory disease, shown to be influenza type B, occurred among personnel of the Johns Hopkins Hospital concomitantly with other localized outbreaks in both civilian and military groups widely dispersed in the United States, Pacific Ocean, Alaska and the Antilles (1). This report is based on clinical observations made at the time of the local epidemic and on subsequent laboratory studies of specimens collected from hospital personnel ill with influenza during this period.

## CLINICAL DATA

*Sources of material:* The group studied includes approximately 600 nurses and dieticians at the Johns Hopkins Hospital, predominantly students. Student nurses are required to report at the onset of any illness to the infirmary where medical service, including hospitalization, is provided. One of the authors (G.R.L.) was personally responsible for the medical care of the group.

*Incidence:* In this group, the epidemic began apparently during the last days of November, 1945, as the onset of the first proved case was November 30. The outbreak reached its maximum by the second week in December, and waned rapidly thereafter. (See Table 1.)

The last two proved cases of influenza "B" began on December 26; several subsequent clinical cases showed no increase of antibodies to either influenza "A" or "B". An estimated 15% of the student nurses were hospitalized with clinical influenza during December.

*Symptoms:* The clinical characteristics of the disease were essentially the same as previously described (2, 3). Clinical data are derived

<sup>1</sup> Supported in part by a grant from the Research Grants Division, National Institute of Health, United States Public Health Service.

<sup>2</sup> From the Department of Bacteriology, School of Hygiene and Public Health, and the Department of Medicine, School of Medicine, The Johns Hopkins University, Baltimore, Maryland.

from 82 cases occurring during November and December, of which 68% of those tested (48 of 71 cases) gave serological evidences of infection with influenza type B.

The mildest symptoms were those of a common cold. The most severe symptoms included prostration, fever sustained at 103-104°F for three or more days, and a prolonged convalescence.

About one-half of the patients described their illness as abrupt in onset, beginning unexpectedly with chilly sensations, or feverishness, shortly followed by prostration, headache, cough, and generalized myalgia. In this group, such upper respiratory symptoms as sore throat, coryza, and nasal congestion appeared late. In the other half, the onset was gradual. Local upper respiratory symptoms predominated.

TABLE 1

*Incidence of clinical cases of influenza by date of onset among student nurses and dietitians at The Johns Hopkins Hospital*

WEEK ENDING	NUMBER OF CASES	PER CENT DISTRIBUTION
12/3	6	7.3
12/10	35	42.7
12/17	21	25.6
12/24	15	18.3
12/31	5	6.1
Total.....	82	100.0

Systemic manifestations appeared only after several days, and resulted in the seeking of medical attention. At the height of the disease, prostration, anorexia, generalized headache, backache, and often aching of all extremities were common in both groups.

Pain back of the eyes, and especially pain on motion of the eyes was commonly described. Photophobia occurred rarely. The throat was described as "dry and scratchy," rather than painful. Cough was common at some stage of the illness, tended to appear late, and was persistent.

*Physical examination:* Few physical abnormalities other than the flushed face, conjunctival injection and pharyngitis were noted. The

oropharynx was remarkably uniform in appearance. It was dull red in color, dry and granular, with loss of the sheen of normal mucous membranes and without exudate or edema. The smaller blood vessels of the soft palate and uvula were often dilated and prominent.

In 8 cases which were shown by serological tests to be influenza Type B, roentgenograms of the chest were taken during the acute stage of the illness. No evidence of pathological changes due to influenza were noted.

*Course of the disease:* The patients were hospitalized as a rule until they had been afebrile for 48 hours, or until they were asymptomatic. The average duration of fever was two and eight-tenths days, of hospital stay, four and eight-tenths days. Weakness, anorexia, and cough

TABLE 2  
*Bacteriological Data*

Throat and Nasopharyngeal Cultures	
Normal Flora.....	48
Possible pathogens predominant.....	12
<i>Staph. aureus</i> .....	4
<i>Str. hemolyticus</i> .....	1
<i>D. pneumoniae</i> .....	3
<i>H. influenzae</i> .....	2
<i>Staph. aureus</i> & <i>Str. hem.</i> .....	1
<i>Staph. aureus</i> & <i>D. pneumoniae</i> .....	1
Total.....	60

were the most persistent of the symptoms, often continuing many days after the temperature had returned to normal. The average loss of working time, including hospitalization, was eight days.

There were no serious complications and no deaths.

*Clinical pathology:* Of 60 throat and nasopharyngeal cultures, 48 yielded normal throat organisms in the usual proportion, and 12 yielded a variety of possible pathogens. In no case did these organisms appear to have any relationship to the cause or severity of the illness.

Leucocyte counts were in the low normal range. Of 32 determinations, the lowest was 3400, the highest 9400, and 75% were between 4800 and 7000 white blood cells per cubic millimeter of blood. The bacteriological data are presented in Table 2.

## LABORATORY INVESTIGATIONS

*Isolation of virus:* A total of 38 throat washings was obtained, nine of which were collected during the first 24 hours of disease. The patient was requested to gargle with two or three 5 ml. of a 50% infusion broth-normal saline mixture, which was stored in sterile test tubes at  $-20^{\circ}\text{C}$ . until tested.

Fertile White Leghorn eggs, incubated at  $38.5^{\circ}\text{C}$ . for 13 days, were inoculated intra-amniotically (6) with 0.4 ml. of unfiltered throat washings containing approximately 200 units of penicillin per milliliter. The inoculated embryos were incubated at  $35^{\circ}\text{C}$ . for 4 days at which time the amniotic fluids were harvested.

These amniotic fluids were tested for the presence of virus by hemagglutination of erythrocytes, using the Salk technic (4). Each amniotic fluid was tested in final dilutions of 1/16, 1/32 and 1/64 in physiological saline, using equal volumes of amniotic fluid dilution and 0.5% washed, normal chicken erythrocytes and 0.7% washed normal guinea pig erythrocytes.

Lungs and tracheas of embryos yielding negative fluids were removed, ground with alundum, and resuspended in the amniotic fluids of the same embryos. This material was used for the next passage. Six passages were made before a specimen was considered negative.

Amniotic fluids shown to contain virus were inoculated into the allantoic sac of 11 day-old embryos and incubated at  $35^{\circ}\text{C}$ . for 48 hours. The embryos were chilled overnight at  $4^{\circ}\text{C}$ . to prevent bleeding. The allantoic fluids were harvested and the viruses were identified by the hemagglutination-inhibition test using known immune serum. Using this technic, 8 strains of influenza "B" virus were isolated from the 38 throat washings collected from nurses and dieticians ill with influenza in late 1945.

Table 3 indicates the day of disease on which the throat washing was collected and the number of specimens from which virus was isolated.

While the numbers in the table are small and no conclusive results may be drawn from them, there is an indication that, using the technic described, virus may be isolated more readily during the first 3 days of disease than at later stages.

*Serological studies:* Two sera from each patient were tested for

change of antibody titre to influenza A and B. The first serum was collected as early in the disease as was feasible, the second usually from 10 days to 5 weeks later. Sera were stored at  $-20^{\circ}\text{C}$ . until used. Both sera from each patient were always tested at the same time.

The method used in detecting a rise in antibodies was the chick cell agglutination-inhibition test, performed essentially as described by Hirst (5). It was simplified by performing the serial dilutions of serum in chicken erythrocyte suspensions, rather than in saline, thus eliminating one step in the procedure. In order to have the same final concentration of erythrocytes in each tube, it is necessary to use a 3% red blood cell suspension in the first tube of each series, and 1.5% suspen-

TABLE 3  
*Frequency of virus isolation by day of disease*

DAY OF DISEASE	NUMBER COLLECTED	THROAT WASHINGS NUMBER POSITIVE
1	9	2
2	8	3
3	12	2
4	3	0
5	3	1
6	0	0
7	1	0
Undetermined	2	0
Total . . . . .	38	8

sions in all others. Agglutination tests were done in tubes 9 mm in diameter. The test was read at the end of 75 minutes, and the end-point determined as the highest dilution of serum which showed 50% or less sedimentation of erythrocytes as compared with a control erythrocyte suspension in saline. Only a rise in antibody titre of at least 4-fold (two tubes) was considered significant. Positive virus and negative serum controls were included in each test.

Virus suspensions were obtained by the intra-allantoic inoculation of 11 or 12-day old chick embryos, using egg adapted PR8 strain of influenza type A virus, and Lee strain of B type virus<sup>1</sup>. The allantoic fluid was harvested after 48 hours incubation and used without further

<sup>1</sup> The authors are indebted to Dr. Thomas Francis, Jr. for strains of these viruses.



treatment. Later, a supply of virus of the same strains, purified by Sharpless centrifugation was used<sup>4</sup>. Four agglutinating units of virus per tube were used routinely in the test (5).

*Results:* Paired sera from 71 patients on whom the diagnosis of influenza was made during November and December, 1945, were tested. Forty-eight (68%) had a 4-fold or greater rise of antibodies to influenza type B and 10 more had a 2-fold rise to influenza type B. None

TABLE 4

*Serum antibody rises of paired sera of patients with clinical influenza, November-December, 1945*

RISE IN TITER	NO. OF PAIRS OF SERA	PER CENT DISTRIBUTION
None	13	18.3
2-fold	10	14.1
4-fold	26	36.6
8-fold	8	11.2
16-fold	7	9.9
>16-fold	7	9.9
Total .....	71	100.0

TABLE 5

*Serum antibody levels of patients from whom Influenza virus was isolated*

ACUTE SERUM LEVEL*	CONVALESCENT SERUM LEVEL*	RISE IN TITER
40	160	4-fold
80	160	2-fold
80	320	4-fold
80	320	4-fold
80	2560	32-fold
160	1280	8-fold
160	640	4-fold
No serum	—	—

showed more than a 2-fold rise to influenza type A. (See Table 4.)  
 The relation of serum antibody to virus isolation may be seen in Table 5.

\*Titers expressed as reciprocals of final dilution.

Table 5 warrants no definite conclusions because of the small number of positive specimens.

<sup>4</sup> Dr. Harold E. Cox kindly furnished this material.

Of the 7 patients with positive throat washings whose corresponding paired sera could be tested, 6 (86%) showed a 4-fold or greater rise of antibodies during convalescence. Of 28 patients with negative throat washings, 20 (71%) showed a similar rise of antibodies.

#### DISCUSSION

Epidemic influenza, type B, appeared in Baltimore during the winter of 1945-46. The occurrence of influenza among the personnel of The Johns Hopkins Hospital afforded the opportunity to make the observations recorded in this paper.

The disease, as observed, was moderately severe, with the usual clinical characteristics. The epidemic was of abrupt onset, and a somewhat more gradual decline. Laboratory evidence, afforded by virus isolations and serological studies, indicated that the epidemic of influenza type B ceased abruptly about four weeks after the onset. Clinically, respiratory disease indistinguishable from influenza occurred with unexpected frequency for at least two more weeks. There is no evidence available which would indicate the etiology of these cases, or of the cases occurring during the epidemic which showed no rise of antibodies to influenza type A or B.

Influenza B virus was isolated 8 times from 38 throat washings. Of the 38 patients whose throat washings were tested, and whose sera were titrated for influenza type B antibodies, 26 showed a 4-fold rise in antibodies, and thus almost certainly had influenza. Presumably they harbored the virus in their respiratory tract at some time during the illness. Of these 26 throat washings 6 yielded virus, or 23%. This percentage could perhaps be improved by using combined nasal and throat washings. Possibly virus isolation can be accomplished with regularity only during the earliest days of the illness. In this study, only one strain was isolated later than the 3d day of disease.

There seems to be no correlation between frequency of virus isolation and either (1) serum antibody level at the time the throat washing was taken, or (2) subsequent rise of antibody to influenza.

The virus strains which have been isolated have not been studied intensively. However, it is apparent that they differ in several aspects from the Lee strain of influenza type B. Hemagglutination titers are lower and tend to diminish with chick embryo passage. Repeated attempts to adapt representative strains to white mice have been un-

successful. However, by serological methods these strains have been shown to be closely related to the Lee strain of influenza type B.

### CONCLUSION

*Summary:* 1. Epidemic influenza type B was prevalent among the nurses and dieticians of The Johns Hopkins Hospital during November and December, 1945. The clinical and laboratory manifestations of the disease among that group are presented. 2. The disease was mild, and involved about 15% of the group. There were no deaths, and no serious complications. The average duration of fever was two and eight-tenths days; of hospital stay, four and eight-tenths days; and of time lost from duty, eight days. 3. Influenza type B virus was isolated from throat washings of 8 of 38 patients by intra-amniotic inoculation of 13 day old chick embryos. 4. Forty-eight of 71 pairs of sera from patients ill with influenza during December, 1945, showed a significant (4-fold) rise of antibodies to influenza type B virus. None showed a significant rise to type A virus.

### BIBLIOGRAPHY

1. FRANCIS, T., JR., SALK, JONAS E., AND BRACE, WM. M.: The Protective effect of vaccination against influenza B, J. A. M. A., 131: 275, May 25, 1946.
2. REYERSBACH, G., LENERT, T. F., AND KUTTNER, A. G.: An epidemic of influenza occurring in a group of rheumatic children concurrent with an outbreak of streptococcal pharyngitis; Clinical and epidemiological observation, J. Clin. Investigation, 20: 289, 1941.
3. STANSFIELD, J. M. AND STUART-HARRIS, C. H.: Clinical study of an outbreak of influenza B during the winter 1942-43, Lancet, 2: 789, 1943.
4. SALK, J. E.: A simplified procedure for titrating hemagglutinating capacity of influenza virus and the corresponding antibody, J. Immunol., 49: 87, 1944.
5. HIRST, G. K.: The quantitative determination of influenza virus and antibodies by means of red cell agglutinations, J. Exp. Med., 75: 49, 1942.
6. BURNET, F. M.: Influenza virus infections of the chick embryo lung, Brit. J. Exp. Path., 21: 147, 1940.

# UNUSUAL PATHOGENICITY OF PASTEURELLA MULTOCIDA ISOLATED FROM THE THROATS OF COMMON WILD RATS<sup>1</sup>

GERALD J. SCHIPPER

*Department of Medicine, The Johns Hopkins University  
School of Medicine, Baltimore, Maryland*

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Our first discovery, that *Pasteurella multocida* is present in the throat of the wild Norway rat, was accidental. When looking for pneumococcus carriers (1), we repeatedly isolated from throat-swabs of wild Norway rats a small, Gram-negative, non-motile rod, which grew well on plain agar, and showed bi-polar staining with Methylene blue. It was finally identified by us, and confirmed by the National Institute of Health, as one of the species of *Pasteurella multocida*.<sup>2</sup> Subsequently, a study was made of the throat-swabs of 102 wild Norway rats caught in the city of Baltimore, and 14 strains of this micro-organism were isolated from 14 different rats.

Since there is no report in the literature of *Pasteurella multocida* being carried in the throats of wild rats, a detailed study of animal pathogenicity, biology, serology, and penicillin sensitivity of the strains was made.

*P. multocida* (sometimes called *P. septica*) has long been held responsible for epidemics of hemorrhagic septicemia in a large variety of animals. The animal pathologist has reported many outbreaks of this disease in horses, cattle, sheep, reindeer, swine, cats, ducks, chickens, rabbits, and mice; all were highly fatal and of short duration. Farmers have named the disease "shipping fever" because of frequent occurrence during transportation of animals. Only one report of hemorrhagic septicemia among wild rats was found. This was an outbreak in the wild rats of Oakland, California, studied in detail by Karl F. Meyer et al. (2). However, in this excellent study, no cross-

<sup>1</sup> This work was supported by a grant from the Dazian Foundation for Medical Research.

<sup>2</sup> We are indebted to Dr. C. L. Larsen for carrying out these tests.

agglutination or cross-pathogenicity tests with other known *Pasteurella multocida* strains were reported.

Moore, in 1895 (3), isolated *P. multocida* from the mucous membranes of the respiratory tract of apparently normal cattle, sheep, swine, dogs, and cats. More recently, Schenk (4) studied in detail strains of *P. multocida* recovered from apparently normal and sick cats, some of which had infected human beings.

Human infections, which have been reported seldom, but are being more often recognized, can be divided into two types. The first type is caused by animal bite, resulting in a local infection, with now and then generalized symptoms. Many cat bites (5, 6, 7, 8, 9, 10), one panther bite (11), one rabbit bite (12), and some dog bites (8) have been reported as causing this type of infection.

The second type of infection has not been connected with any known, direct cause. It may present a wide variety of clinical symptoms, ranging from long standing recurrent chills and fever to isolated cases of pleuritis and meningitis. Such infections have been reported mainly in Europe (13), and never in the United States.

The large group of *Pasteurella* organisms, causing hemorrhagic septicemia in animals, has been divided into various species, according to the animal from which each was isolated. Thus various names as *P. avicida*, *P. bovisepctica*, *P. suisepctica*, *P. lepiseptica* have been used. Evidence has been accumulating that this zoological classification is meaningless since cross-pathogenicity tests and cross-agglutination tests by many workers (14, 15, 16), as well as cultural and biochemical similarities, showed that all the strains isolated from different animal species were nearly identical. Recently, Rosenbusch and Merchant (17) studied extensively 114 strains from many different animals and could not differentiate the strains by serological tests. Therefore, the name proposed by Kitt in 1895, *P. multocida*, has been adopted for all animal strains.

#### EXPERIMENTAL

##### *Isolation of Strains of Pasteurella Multocida*

The 102 wild Norway rats used for the isolation of the 14 recovered *Pasteurella* strains were made available to us by Dr. Curt P. Richter of the Psychobiological Laboratory of the Johns Hopkins Hospital. They were trapped in different parts of the City of Baltimore, Mary-

land, and at the time of throat-swabbing had not been held longer than 4 days in the laboratory. With the aid of the wiresock, first described by Emlen (18), these wild rats could be handled very easily. After the rat had been secured in a sock, the upper and lower incisors were held apart by the loop of the end of a wire brush handle. A cotton swab was then easily inserted into the pharynx.

Every throat-swab was placed immediately into about 2 cc. of beef infusion broth and used the same day for plating on rabbit-blood-agar and for injection of 0.25 cc. intraperitoneally into one adult Swiss mouse. The remainder of each specimen was kept in a dry ice box at  $-78^{\circ}\text{C}$ . for later studies.

Every rabbit blood agar-plate was incubated at  $37^{\circ}\text{C}$ . for about 20 hours, and a colony, morphologically suggestive of *P. multocida*, was picked in beef infusion-broth and further biochemical studies were made. Diphtheroids, streptococci and proteus were abundant on the blood plates.

From every mouse which died within 4 days after inoculation, a culture of the tailblood and peritoneal exudate was taken. All mice dying within that period yielded pure cultures of *P. multocida*, except three from which pure cultures of streptococci were obtained and an additional five from which mixed cultures of proteus and suggestive pasteurilla were obtained. Repeated attempts to get rid of proteus by intraperitoneal mouse-inoculations failed.

A total of 14 strains were isolated by the biological method of mouse-inoculation and could also be identified morphologically on the blood plate cultures of the original throat-swabs. Their similarity by other biochemical tests was later established.

Several blood plate cultures from throat swabs which did not kill mice yielded strains suggesting *P. multocida*, but were not used for further studies since they were not virulent for mice.

The 14 strains isolated from 102 rats and used for further study in this work were, therefore, specific mouse-virulent strains and did not necessarily represent the true percentage of carriers of *P. multocida* in the rats. Moreover, five strains isolated from mouse-cultures were overgrown with proteus, as mentioned above, and were discarded.

Twenty original throat-washings, which showed diphtheroids on the blood agar plate, were streaked on Loeffler slants and colonies, suggestive of *C. diphtheriae* picked for growth on tellurite medium.

Colonies on the tellurite medium were selected because of typical metal-like appearance, and three pure cultures were finally selected which were highly suggestive of *C. diphtheriae*. However, subsequent tests in guinea pigs showed them to be avirulent strains.

### *Animal Pathogenicity*

All 14 strains of *P. multocida* were tested for virulence in mice and rabbits, and two of our first isolated strains, WR2b and WR38, were tested in a large number of other animal species.

The pathogenicity of the other 12 strains for mice and rabbits, and of strains WR2b and WR38 for rabbits and other animal species was tested in each case by injecting intraperitoneally 0.5 cc. of a  $10^{-2}$  dilution in beef broth of an 18 hour beef broth culture of the strain. From animals which died, a heart or tail blood culture was taken and only animals with a positive culture were accepted as having succumbed to the inoculum. All but two strains of *P. multocida* included in this study killed mice and rabbits within 24 hours after inoculation.

The virulence of strains WR2b and WR38 was tested in mice by the intraperitoneal injection of 0.5 cc. of serial 10-fold dilutions of an 18 hour broth culture. All mice inoculated with dilutions ranging from  $10^{-0}$  up to  $10^{-6}$  died in 12-24 hours; the mice with the highest dilutions succumbing last.

Inocula of 0.5 cc. of a  $10^{-2}$  18 hour broth culture of strains WR2b and WR38 were found to kill the following animals within 24 hours after intraperitoneal injection: 2 guinea pigs, 2 hamsters, 4 Cotton rats, 4 Alexander rats, 3 chickens. Strain WR2b was similarly tested in a cat and a dog, and killed each in about 12 hours.

Following subcutaneous inoculation into rabbits, *P. multocida* produced death within 3 to 4 days. On autopsy, characteristic gross lesions were found consisting of serous exudates in the peritoneal, pericardial and pleural cavities and occasional petechial hemorrhages in the lungs.

Having found the unusual virulence of *P. multocida* for many animals, including a dog, the question arose whether a rat, carrying *P. multocida* in his throat, would be able to infect a susceptible animal by biting.

For this purpose a small part of the belly of a dog was shaven, the

skin folded, and two Norway rats, (WR67, 81) carrying *Pasteurella* in the throat were allowed to bite through the skin till some blood appeared. The dog was kept under observation for 14 days but did not show any sign of illness in this time, so observations were discontinued. This forgotten dog, however, was found dead in its cage 23 days after the original rat bites. On autopsy, pathological changes were found only in the lungs, namely, tiny diffusely scattered haemorrhagic areas. The peritoneal cavity appeared normal and no abscesses were found. Cultures were taken from the peritoneum, heart blood and lung. Only the lung culture showed growth, a mixed culture of streptococci and a Gram-negative bipolar rod, which was subsequently identified as *P. multocida*.

To our surprise, Norway rats and hooded or albino rats were found to be resistant even to the intraperitoneal inoculation of 0.5 cc. of undiluted culture. This was confirmed during a six month period of repeated trials on a total number of 16 tame rats and 15 Norway rats, several of the latter carrying *P. multocida* in their throats. Also, intranasal and subcutaneous inoculations on the abdominal wall or at the root of the tail, to which mice were susceptible, did not seem to make the rats sick. All rats were kept under observation for a 20 day period.

To investigate further this non-susceptibility of the Norway rat and the tame rat, a series of four experiments was carried out:

a. Norway rats, Cotton rats, and mice were inoculated intraperitoneally with undiluted 18 hour broth cultures of strains WR38 and WR48, and blood cultures were taken at varying intervals. In Table I it will be seen that the blood cultures from mice and Cotton rats were positive at 4, 6, and 8 hours after inoculation, whereas the cultures from the Norway rats were sterile.

b. The pooled sera of Norway rats carrying *P. multocida* and the pooled sera of Norway rats from which this microorganism was not cultured were tested for agglutinins against strain WR38. The sera was tested both unheated and following inactivation at 56°C. for 30 minutes. As controls, the serum of a normal rabbit and of a rabbit immunized, as described later, against this strain of *P. multocida* were included. Serial twofold dilutions of the sera in physiological saline were made and mixed with an equal volume of the antigen.



The antigen used in this test was a suspension in physiological saline of heat-killed, saline washed, bacterial cells which had been grown in beef broth 24 hours at 37°C. The test was read after incubating the mixture at 41°C. for 24 hours and then keeping it in the icebox at about 4°C. overnight. It will be seen in Table II that no agglutination was produced by the pooled rat sera or the normal rabbit serum

TABLE I  
*Invasiveness of Pasteurella multocida Strains*

SPECIES INOCULATED	PASTEURELLA STRAIN	BLOOD CULTURE AFTER:		
		4 hrs.	6 hrs.	8 hrs.
Mouse. ....	WR38	+	+	+
Mouse. ....	WR48	+	+	+
Norway rat. ....	WR38	-	-	-
Norway rat. ....	WR48	-	-	-
Cotton rat. ....	WR38	+	+	+
Cotton rat. ....	WR48	+	+	+

TABLE II  
*Agglutination of Pasteurella multocida*

SERUM	SERIAL DILUTIONS OF SERUM					
	1/80	1/160	1/320	1/640	1/1280	1/2560 (final)
Carrier-rat pool. ....	-	-	-	-	-	-
Non-carrier rat pool. ....	-	-	-	-	-	-
Immune rabbit. ....	+	+	+	+	+	+
Normal rabbit. ....	-	-	-	-	-	-

+ = agglutination

- = no agglutination

in dilution of 1/80 or greater. Agglutination was observed in the homologous immune rabbit serum in a dilution of 1:2560.

c. Two rats and two mice were simultaneously inoculated intraperitoneally with 0.25 to 0.50 cc. of a  $10^{-2}$  dilution of a 10 hour broth culture of strain WR38. At different intervals after inoculation the peritoneal cavity of each animal was thoroughly washed with 1 to 2 cc. of sterile broth. From each peritoneal washing a smear was

made, stained with Methylene blue, and studied microscopically, for phagocytosis and number of organisms. Each washing was also cultured on rabbit blood agar plates, and studied for growth after 18 hours incubation at 37°C. It will be seen in Table III that 3 hours after inoculation the smears from the rat and mouse peritoneal cavities were indistinguishable. At 10 hours, however, phagocytosis was much more prominent in the case of the rat though the culture was positive for each species. The smear from the rat at 24 hours showed no micro-organisms and cultures were sterile, whereas the mice had succumbed to the infection.

TABLE III

*The Fate of Pasteurella multocida in the Peritoneal Cavity of Mice and Rats*

HOURS AFTER INTRAPERITONEAL INOCULATIONS	PERITONEAL WASHING OF RAT	PERITONEAL WASHING OF MOUSE
3 hours later	Phagocytosis + Many organisms Culture positive	Phagocytosis + Many organisms Culture positive
10 hours later	Phagocytosis ++ Few organisms Culture positive	Occasional phagocytosis Numerous organisms Culture positive
24 hours later	No organisms observed Culture negative	Mouse dead Smear teeming with organisms, showing nice bipolarity. Culture positive.

d. Serum of three Norway rat carriers of *P. multocida* (WR47, WR48, WR49) was pooled. A separate pool was made of the serum from three other Norway rats from whom the microorganism was not recovered. With these two serum pools, neutralization experiments were carried out in mice. An inoculum of 0.25 cc. of 10<sup>-5</sup> dilution of an 18 hour broth culture of strain WR48 (10 M.L.D.) was injected subcutaneously into four mice, two of which had received 0.25 cc. of each serum pool intravenously shortly before. All the mice died within 24 hours after inoculation, and *P. multocida* was recovered by culturing the tail blood. This rigorous test then failed to demonstrate any appreciable difference in neutralizing antibody in the two types of rat serum.

## Biology

To identify the *Pasteurella* strains recovered from the throat-swabs of wild Norway rats, a study was made of the morphological and cultural characteristics and the findings were compared with those of

TABLE IV  
*Biological characteristics of P. multocida strains*

P. STRAIN	HOST ORIGIN	FERMENTATION REACTION																							ROSENBLUSCH-MERCHANT GROUPS		
		OF RABBIT BLOOD	POTATO	DESODIUMED AGAR	INDUCTION	LITMUS MILK	HEMIC	BIPO.	MOT.	GROW.	INDO.	CHAM.	CATY.	LACT.	DEX.	SUCK.	LEVO.	FORB.	GALA.	RAFF.	ABAR.	DULC.	XYLOSE	MALTOSE		BILE SOLUBILITY	MOUSE PATHOGENICITY
WR2b	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I
WR3b	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I
WR7	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I
WR9	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I
WR11	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I
WR25	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I
WR37	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?
WR38	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?
WR45	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?
WR47	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	II
WR48	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?
WR49	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I
WR67	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?
WR81	Rat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?
RT I	Rabbit	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	II
Fl I	Fowl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I
Ct I	Cat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	II
PM195	Cow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P. hemolytica
JSB I	Sheep	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	II

0 = Not done.

- = Negative

+ = Positive or acid.

*Pasteurella* strains recovered from different animal species. The results of all the following tests are shown in Table IV.

*Strains of Pasteurella:* Strain Ct. I was isolated from the throat of a wild cat, caught by Dr. D. Davis of the Department of Rat Ecology, of the Johns Hopkins School of Hygiene; the animal was known to feed on wild rats. Strains Rt. I and Fl. I were kindly given us by

members of the Department of Bacteriology of the Johns Hopkins School of Medicine. Finally, Dr. F. A. Merchant of the Department of Veterinary Hygiene, Iowa State College, supplied us with Strains P.M. 195 and J.S.B. I. The 14 strains of *P. multocida* recovered from Norway rats by the methods described above were employed in addition.

*Hemolysis:* The capacity of the strains to hemolyse rabbit blood was tested by incubating them for 48 hours on agar plates containing 10% rabbit blood at 37°C. Only the bovine strain, PM195, caused hemolysis, and by its fermentation of lactose and maltose proved to be a true *Pasteurella hemolytica*. According to most workers, this is an atypical *Pasteurella multocida* and apparently deserves a separate classification.

*Bipolarity:* Bipolarity was observed in smears of 18 hour broth cultures stained for 2 minutes with Methylene blue. This simple staining method was satisfactory for demonstrating bipolarity and, moreover, can be used easily in any routine diagnostic laboratory. Bipolar staining was especially clear after animal passage, and in our belief, doubtful bipolarity may be accentuated by animal passage.

*Motility:* Motility was studied in a hanging drop preparation of 18 hour broth cultures. No strain was found to be motile.

*Growth on Potato and Desoxycholate media:* All strains grew well in plain infusion broth and on rabbit blood agar plates. None of the strains tested showed growth on plain potato or desoxycholate agar plates.

*Indol Production:* All strains formed indol in peptone-water at 48 hours incubation, but there was a marked difference in indol production among the various strains. Strain Fl. I gave only a slightly positive reaction after 4 days of incubation.

*Litmus Milk:* Change in color was read after 10 days incubation. Only Strain PM195 gave a definite color change, becoming red after 3 days incubation.

*Catalase:* The presence of catalase was demonstrated by the release of oxygen from hydrogen peroxide poured over an 18 hour culture on a plain agar slant.

*Bile Solubility:* Two drops of ox bile were added to 1 cc. of 18 hour infusion broth culture of each strain. No strain was bile soluble.

*Pathogenicity for mice and rabbits:* Each strain was tested for pathogenicity as described above. Only Ct. I and P.M.195 were found to be non-pathogenic for mice.

*Fermentation reactions:* The ability of each strain to ferment various carbohydrates was tested by inoculating several drops of an 18 hour broth culture into broth containing 1% of the test-substance and incubating at 37°C. The production of acid and gas was observed, at intervals, for 15 days. None of the strains produced gas but all strains produced acid slowly in 4 to 10 days on certain substrates. Strain P.M.195 produced acid much more rapidly.

*Rosenbusch-Merchant Groups.* Rosenbusch and Merchant (17), when studying 114 strains of *Pasteurella multocida*, were able to divide these strains into two groups on the basis of fermentation reactions and serological characteristics. They found that arabinose, dulcitol, and xylose fermentations were sufficient to set up classification of these groups. Group I fermented arabinose and dulcitol, but not xylose; it included some strains from all animal species tested. Group II fermented only xylose; it also included some strains from all animal species tested, except birds. Another small group did not give uniform fermentation reactions; it consisted mainly of ovine-bovine strains, and cross agglutinated poorly with the other groups. As will be seen in Table IV, six of our Norway rat strains cannot be classified in any of the Rosenbusch-Merchant groups. Of the other rat strains, 5 belong to Group I and one conforms to Group II. It should be noted that insufficient fermentation tests were carried out on two of the rat strains to permit classification by this method. This was due to their loss as a result of our failure to realize the necessity of frequent (preferably weekly) passage through broth into fresh blood agar. It was found inadvisable to preserve cultures, without passage, in the icebox for longer than 10 days.

### Serology

Cross-agglutination of *P. multocida* strains was studied. All strains listed in Table IV were included. The results of this comparative study are shown in Table V.

*Preparation of Anti-serum:* Specific anti-sera were prepared from three rat strains (WR2b, WR38, WR48) and one fowl strain (Fl. 1).

Rabbits were inoculated intravenously with heat-killed vaccine in increasing dosages over a period of 30 days. Each rabbit received a total dose of 15 cc. and was bled the first and thirtieth day. The serum of the first bleeding was taken as the normal rabbit serum control. The vaccine consisted of a suspension in physiological saline of the bacterial cells obtained from 20 hour infusion broth cultures.

TABLE V

*Agglutination tests of Pasteurella multocida strains with specific rabbit antisera*

PASTEURELLA STRAIN	HOST ORIGIN	AGGLUTINATION TITRE BY SERUM				NORMAL RABBIT
		WR2b	WR38	WR48	Fl 1	
WR2b	Norway rat	0	0	0	0	0
WR3b	Norway rat	0	1/1280	0	0	Neg.
WR7	Norway rat	1/1280	1/2560	1/320	1/2560	Neg.
WR9	Norway rat	1/1280	1/2560	1/320	1/2560	Neg.
WR11	Norway rat	1/1280	1/1280	1/320	1/2560	1/40
WR25	Norway rat	1/1280	1/1280	1/320	1/1280	Neg.
WR37	Norway rat	1/2560	1/2560	1/320	1/1280	1/40
WR38	Norway rat	1/2560	1/5120	1/320	1/2560	Neg.
WR45	Norway rat	1/1280	1/1280	1/640	1/2560	Neg.
WR47	Norway rat	1/2560	1/640	1/2560	1/320	Neg.
WR48	Norway rat	1/1280	1/640	1/5120	1/1280	Neg.
WR49	Norway rat	1/1280	1/1280	1/640	1/1280	Neg.
WR67	Norway rat	1/1280	1/2560	1/640	1/2560	Neg.
WR81	Norway rat	1/1280	1/2560	1/320	1/2560	Neg.
Rt. 1	Rabbit	1/1280	1/2560	1/2560	1/320	Neg.
Fl. 1	Fowl	1/1280	1/1280	1/320	1/5120	Neg.
Ct. 1	Cat	1/2560	1/2560	1/640	1/640	Neg.
PM195	Cow	Neg.	Neg.	Neg.	Neg.	Neg.
JSB 1	Sheep	1/640	1/640	1/160	1/320	1/40

0 = not done.

Neg. = negative.

The cells were washed three times, concentrated ten times, killed by heating one-half hour at 63°C., and tested for sterility.

*Preparation of Antigen:* Initially, 20 hour agar plate cultures were used, but later 20 hour rabbit blood agar plates with 1% added sucrose were found to yield richer, more mucoid growth. The bacterial cells washed from the plates with sterile saline were killed with 0.5% formalin. Antigen prepared from the rabbit blood agar plates with

1% sucrose is supposed to be more stable and exhibited less auto-agglutination.

*Agglutination Technique:* Serial dilutions of the different anti-sera, ranging from 1/40 to 1/10,240, were prepared in physiological saline and were incubated with each antigen in a water bath of 41°C. as described above under animal pathogenicity. Readings were made after 24 to 48 hours incubation, and again after standing over-night in the icebox. Normal rabbit serum controls were run, and each test was repeated twice. Whenever auto-agglutination occurred, fresh antigen was prepared and the test repeated.

*Results:* As shown in Table V, homologous agglutination titers ranged from 1/2560 to 1/5120, final dilution of serum. Normal rabbit serum never agglutinated in a dilution above 1/40. The bovine strain did not agglutinate with any anti-sera. It will be seen in Table V that all other strains cross-agglutinated.

The antigenic similarity of all the strains tested, with the exception of the cow strain PM195, is apparent. There seem to be minor differences between certain of the strains, but these antigenic differences do not correlate with the differences noted by the fermentation tests recorded in Table IV. It is of interest that the 6 strains (WR37, WR38, WR45, WR48, WR67, WR81) which could not be placed in one of the Rosenbusch-Merchant groups by fermentation tests, nevertheless are antigenically similar to the other strains tested. This is contrary to the findings of these workers (17) and suggests that these 6 rat strains represent a separate group different from any by them encountered.

Sixty human sera, several obtained during convalescence from patients with fever of unknown origin, were tested for agglutination with *P. multocida* antigens WR38 and Fl. 1. Except for an occasional 1/40 titre, the agglutinations were negative. In addition, convalescent sera from three patients with Brucellosis, 2 patients with Tularemia, and 2 with *H. influenzae* infection were similarly tested and found to be negative with the *P. multocida* antigen even though these sera showed high agglutinin titers with the homologous antigens. Moreover, *P. multocida* specific rabbit anti-serum was tested for agglutination with *B. melitensis*, *P. tularensis* and *H. influenzae* antigens, with negative results.

*Penicillin Sensitivity*

One of our *P. multocida* strains (Strain WR38), isolated from the throat-swab of a wild Norway rat, was tested *in vitro* for sensitivity to streptomycin and penicillin. This Pasteurella strain was found to be rather resistant to streptomycin bacteriostasis since 4.5 units/cc. inhibited growth. It did show unexpected sensitivity to penicillin bacteriostasis, growth being inhibited by as little as 0.1 unit per cc.

As extreme sensitivity to penicillin is rather unusual for a Gram-negative micro-organism, penicillin sensitivity tests were done of all the other *P. multocida* strains available and the same unusual sensitivity was found. Certain other Gram-negative rods, which might be confused with *P. multocida*, were similarly tested. All tests were done at least twice.

The method used to test penicillin sensitivity was to inoculate each of a series of tubes of fresh beef infusion broth containing varying amounts of penicillin with an equal volume of the broth containing a  $10^{-3}$  dilution of a 20 hour broth culture of each bacterium. The final concentrations of penicillin ranged from 0.1 unit to 0.9 units per cc. of medium with increments of 0.1 unit per tube. The tubes were incubated at 37°C. and the presence or absence of growth was noted at 24 and 48 hours, subcultures being made in case of doubt. The penicillin employed was the fresh crystalline sodium salt. (Merck)

It will be seen in Table VI that all nineteen of the *P. multocida* strains tested were sensitive to 0.2 units of penicillin per cc. or less. All of the other Gram-negative species tested were resistant to 0.9 units or more. Meyer (19) found that some strains of *P. pestis* were killed by 0.02 units of penicillin, whereas 4 strains of *P. pseudotuberculosis* isolated from different animal hosts were resistant to as much as 1.5 units of penicillin per cc. One starts wondering if it is only a coincidence that of all Gram-negative rods only *P. pestis* and *P. multocida* show this peculiar hypersensitivity to penicillin *in vitro*. Recently a *P. multocida* strain, isolated from wild ducks, was reported to be sensitive to 0.08 units of penicillin per c.c. (42).

## DISCUSSION

This study has shown that a certain percentage (14%) of wild Norway rats studied carried virulent *P. multocida* organisms in their



throats. The strains recovered were found to be unusually virulent for a wide variety of animals. The implications of these findings are twofold, (1) concerning the epidemiology of hemorrhagic septicemia in animals and (2) concerning the epidemiology of human cases of pasteurellosis.

TABLE VI  
*Penicillin Sensitivity of Pasteurella multocida in Vitro*

STRAIN	HOST ORIGIN	UNITS OF PENICILLIN PER CC. OF MEDIUM									
		.9	.8	.7	.6	.5	.4	.3	.2	.1	0.
<i>P. multocida</i> WR2b	Rat	—	—	—	—	—	—	—	—	—	+
<i>P. multocida</i> WR3b	Rat	—	—	—	—	—	—	—	—	—	+
<i>P. multocida</i> WR7	Rat	—	—	—	—	—	—	—	—	—	+
<i>P. multocida</i> WR9	Rat	—	—	—	—	—	—	—	—	+	+
<i>P. multocida</i> WR11	Rat	—	—	—	—	—	—	—	—	+	+
<i>P. multocida</i> WR25	Rat	—	—	—	—	—	—	—	—	+	+
<i>P. multocida</i> WR37	Rat	—	—	—	—	—	—	—	—	—	+
<i>P. multocida</i> WR38	Rat	—	—	—	—	—	—	—	—	—	+
<i>P. multocida</i> WR45	Rat	—	—	—	—	—	—	—	—	—	+
<i>P. multocida</i> WR47	Rat	—	—	—	—	—	—	—	—	—	+
<i>P. multocida</i> WR48	Rat	—	—	—	—	—	—	—	—	+	+
<i>P. multocida</i> WR49	Rat	—	—	—	—	—	—	—	—	—	+
<i>P. multocida</i> WR67	Rat	—	—	—	—	—	—	—	—	—	+
<i>P. multocida</i> WR81	Rat	—	—	—	—	—	—	—	—	+	+
<i>P. multocida</i> Rti	Rabbit	—	—	—	—	—	—	—	+	+	+
<i>P. multocida</i> FII	Fowl	—	—	—	—	—	—	—	—	—	+
<i>P. multocida</i> Cti	Cat	—	—	—	—	—	—	—	—	—	+
<i>P. multocida</i> PM195	Cow	—	—	—	—	—	—	—	—	+	+
<i>P. multocida</i> JSBI	Sheep	—	—	—	—	—	—	—	—	+	+
<i>H. influenzae</i> 1	Human	+	+	+	+	+	+	+	+	+	+
2	Human	+	+	+	+	+	+	+	+	+	+
3	Human	+	+	+	+	+	+	+	+	+	+
4	Human	+	+	+	+	+	+	+	+	+	+
<i>Salm. pullorum</i>	Chicken	+	+	+	+	+	+	+	+	+	+
" <i>suipestifer</i>	Human	+	+	+	+	+	+	+	+	+	+
<i>Paracolon bacillus</i>	Human	+	+	+	+	+	+	+	+	+	+
<i>H. bronchisepticus</i>	Rabbit	+	+	+	+	+	+	+	+	+	+

Growth = +.

No growth = —.

Hemorrhagic septicemia occurs in epidemic form in a wide variety of animals, and its ravages on wild and domestic animal life have long been recognized. Outbreaks of fowl cholera (hemorrhagic septicemia) among chickens have never been explained by the presence of chronic

carriers (20). In flocks studied by the Bureau of Animal Industry of the U. S. Department of Agriculture, it was found (21) that the incidence of fowl cholera was no higher in those flocks having chronic carriers than in those without carriers. Our study has incriminated the rat as a reservoir for virulent *P. multocida*, and considering the close contact of this animal with chickens, the wild rat may well be the missing link in the epidemiology of fowl cholera. Norway rats are known as carnivorous scavengers, and may acquire the carrier state by feeding on animals having died of hemorrhagic septicemia. It can, furthermore, be postulated that the wild Norway rat may play an important role in the epidemiology of hemorrhagic septicemia of other animals. For instance, as yet unexplained is the fact that cats and dogs are sometimes found dead in alleys after having chased rats (22). As was shown in our study, a small inoculum of *P. multocida* may kill these animals by acute septicemia overnight. The possibility of rat bite should always be entertained in the sudden death of domestic animals.

Human cases of pasteurellosis have been ascribed to cat bites, dog bites, etc. (see Appendix), but never to rat bites or contact with rats. How intimate the contact of the wild Norway rat can be with human beings was borne out in a recent study (23) in Baltimore; 95 patients with rat bites were admitted to the Johns Hopkins Hospital alone from an area of less than two square miles during a four year period. We believe that the potentialities of the spread of human pasteurellosis by the rat are great, and that careful search for *P. multocida* should be made in all cases of sickness in human beings following contact with rats.

The extreme sensitivity of the strains of *P. multocida* to penicillin *in vitro* as shown in this study is promising for the control of the disease and may, moreover, prove to be a valuable aid for the identification of this micro-organism. In the belief of many workers (19, 24, 25), *P. multocida* is not infrequently mistaken for *H. influenzae* in the routine laboratory, and in a summary of the literature of human cases of Pasteurellosis at least 5 were initially misdiagnosed as *H. influenzae* infections. (See Appendix). Certain strains of *H. influenzae* could easily be confused with *P. multocida* because of similar biochemical characteristics. Moreover, *H. bronchisepticus*, which is known to cause acute respiratory disease in rabbits and dogs and a

whooping cough-like syndrome in children (26), may be confused with *P. multocida* for the same reasons. Our study has shown that both *H. influenzae* and *H. bronchisepticus* are significantly more resistant *in vitro* to penicillin than *P. multocida*. We therefore suggest the use of penicillin sensitivity *in vitro* as an additional laboratory method for the differentiation of *P. multocida* from other Gram-negative rods.

Our study has borne out previous observation (27) that the laboratory rat is resistant to infection by *P. multocida*, and has shown that this is also true for the wild Norway rat. It is of interest that other species of rats such as the Cotton rat and Alexander rat are highly susceptible to infection by *P. multocida*. We have failed to demonstrate neutralizing and agglutinating antibodies in the sera of the Norway, hooded or albino rat, but have succeeded in showing rapid phagocytosis in these species. Other factors which may be involved in the natural resistance of these animals have not yet been elucidated.

Our studies of the 14 strains of *P. multocida* isolated from the throats of wild Norway rats have shown that about half could be classified according to the Rosenbusch-Merchant grouping (17); i.e. by biological and serological reactions. Six of our strains defied classification into their groups on the basis of fermentation reactions. Contrary to the findings of the above workers these strains showed antigenic similarity to the strains of the two main groups. Five of these six strains fermented arabinose and xylose but not dulcitol, and thus may represent a separate group. The rest of our strains fell into Group I. It is interesting to note that in the review of twenty human cases of Pasteurellosis (see Appendix), nearly all the strains of *P. multocida* recovered belonged to Group II, which contains strains from all animal species except birds.

On the basis of this study, certain recommendations are made for the routine identification of *P. multocida*. 1. Fermentation reactions are not conclusive for the identification of *P. multocida*. 2. Animal passage will often bring out latent bipolarity. 3. Intraperitoneal inoculation of mice as used in routine laboratories is not a good criterion, as many Gram-negative organisms will kill mice by acute septicemia. 4. Subcutaneous inoculation of *P. multocida* into rabbits will produce a typical pathological picture of hemorrhagic septicemia, valuable for identification of the microorganism. 5. Since almost all

the strains of *P. multocida* studied showed antigenic similarities to each other but differed from the other bacterial species tested, specific antisera may be useful in their identification. 6. Penicillin sensitivity may be used as a rapid *in vitro* test for differentiating *P. multocida* from other Gram-negative rods.

#### SUMMARY

1. Among 102 wild Norway rats caught in Baltimore, 14 carried virulent *P. multocida* in their throats.

2. These strains of *P. multocida* were unusually virulent for a wide variety of animals, while the Norway rat and laboratory rat were completely resistant. Further studies were made to elucidate the mechanisms of this resistance.

3. Detailed study of the bacteriology and serology of the 14 recovered strains were made, and their classification discussed.

4. All 14 strains and also strains of *P. multocida* from other laboratories were sensitive *in vitro* to less than 0.2 units penicillin/cc. of media.

5. It is suggested that the penicillin sensitivity test *in vitro* be used as an additional method for differentiating *P. multocida* from other Gram-negative rods.

6. A comprehensive summary is included of clinical and bacteriological data concerning human cases of pasteurellosis reported in the world literature from 1930-1947.

7. Evidence is presented that *P. multocida* may be easily confused with other Gram-negative microorganisms, hence it is urged that diligent search for *P. multocida* be made in all cases of obscure infection in animals or human beings.

#### APPENDIX

Tables VII and VIII present clinical and bacteriological data, respectively, from reports of human pasteurellosis collected from the world literature from 1930-1947. Of more than thirty cases reported since 1930, 21 have been selected as showing the most convincing evidence of true pasteurellosis. An attempt has been made to review in the tables only reports based on good bacteriological studies. Early reports of human Pasteurella infection prior to 1930, have been summarized (28). For this reason, and because 1930 marks the

TABLE VII

Clinical data from reports of human pasteurellosis. (A summary of published cases from 1930-1947.)

REPORT %	AUTHOR	COUNTRY	YEAR	NO. OF CASES	MECHANISM OF INFECTION	TYPE OF DISEASE	PASTURELLA ISOLATED FROM	DURATION OF ILLNESS	OUTCOME
1	Hundeshagen (29)	Germany	1919	1	Unknown	Pleurisy	Pleural exudate repeatedly	4 months	Complete recovery
2	Kapel et al (5)	Denmark	1930	1	Cat-bite	Infection of hand	Abscess	1 month	Permanent dysfunction of finger
3	Rimbaud (32)	France	1931	1	Unknown	Pleurisy	Pleural exudate	2 months	Complete recovery
4	Slecht (36)	Germany	1931	1	Consumption of infected chicken	Severe hepatitis with icterus	Duodenal washings repeatedly	4½ months	Complete recovery
5	Levy-Bruhl (37)	France	1934	1	Rabbit-muscle used as hemostat	Meningitis	Cerebrospinal fluid	3 days	Death
6	Rivoalen (11)	Morocco	1936	1	Panther-bite	Septicæmia	Blood and abscess	4 months	Complete recovery
7	Foerster (38)	Germany	1938	1	Unknown	Progressive lung disease	Pleural exudate and postmortem	11 years	Death
8	Peltier et al (39)	France	1938	1	Unknown	Acute and fatal septicæmia	Blood and post mortem	10 days	Death
9	Mulder (24)	Holland	1938	1	Unknown	Chronic and the	Sputum	Several years	?

10	Regamey (25)	Switzerland	1938	1	Latent Sinus-infection, to CNS after trauma	Late post-traumatic meningitis	Repeatedly from cerebrospinal fluid	1 month	Complete recovery
11	Plette (33)	Holland	1938	1	Unknown	Bilateral broncho-pneumonia with pleural & pericardial effusion	Pleural exudate & also post-mortem	Several weeks	Death
12	le Chuiton (34)	France	1939	1	Cranial fracture	Meningitis	Cerebrospinal fluid	1 month	Complete recovery
13	Weber (6)	Germany	1941	10	Cat-bite	Infection of extremities	Abscesses	1 week-3 months	One amputation of forearm
14	Boisvert et al (12)	U. S. A.	1941	1	Rabbit-bite	Infection of hand & forearm	Abscess	1½ months	Recovery
15	Pestana et al (40)	Brazil	1941	2	Unknown	Pleurisy	Pleural exudate	1 month	Recovery
16	Allin (7)	Canada	1942	2	Cat-bite	Infection of extremities	Abscess	Subclinical 11 months	Recovery
17	Reinders (35)	Holland	1943	1	Latent infection flaring up after trauma (Operation)	Meningitis	Cerebrospinal fluid	1 month	Good recovery

TABLE VII—*continued*

REPORT #	AUTHOR	COUNTRY	YEAR	NO. OF CASES	MECHANISM OF INFECTION	TYPE OF DISEASE	PASTURELLA ISOLATED FROM	DURATION OF ILLNESS	OUTCOME
18	Ludlam (41)	Scotland	1944	1	Unknown	Appendicular abscess	Pus of abscess	Several weeks	Recovery
19	Allot et al (8)	England	1944	7	Cat and dog bite	Infection of extremities with sub-acute osteomyelitis	Abscesses	Weeks to several months	Recovery
20	Hansmann et al (9)	U. S. A.	1945	2	Cat bite	Infection of extremities	Abscesses	1 week to months	Recovery
21	Cooper et al (10)	England	1945	1	Cat bite	Infection of hand	Abscess	6 weeks	Recovery
22	Thjotta et al (43)	Norway	1946	1	Unknown	Pneumonia with empyema	Pleural exudate	?	Recovery

beginning of reports of human pasteurellosis caused by animal bites, (5), our summary extends from that date. One additional case, (29) reported in 1919 by Hundeshagen in Germany is included because it is regarded as the first well-reported case in the literature, and because it occurred during the influenza pandemic of 1919. During this pandemic, many Gram-negative Pasteurella-like organisms, showing bipolar staining, were isolated from cases of pneumonia (30, 31). Identification was never conclusively established, although most cases were classified as due to *H. influenzae*. As is known, *H. influenzae* may closely resemble *P. multocida* in its bacteriological characteristics, and it is possible that more cases of Pasteurella infection were overlooked during the pandemic. Also, in our summary, it will be found that the initial diagnosis of *H. influenzae* infection was made in at least five cases (cases 24, 25, 29, 32, 33) subsequently proven to be infections by *P. multocida*.

The varying mechanisms of infection and the wide variety of clinical manifestations will be noted in Table VII. It is of considerable interest that *P. multocida* may apparently lie dormant in tissue for months, giving rise to an acute infection after traumatization of the sub-acutely infected tissue (7, 25, 34, 35).

Table VIII presents the bacteriological data of the strains of *P. multocida* isolated from these human cases of pasteurellosis. It should be mentioned that all strains were Gram-negative, non-hemolytic, non-motile, and showed growth on plain agar, did not ferment lactose, and produced indol. Bipolarity was demonstrated in some instances only after animal passage. Odor and  $H_2S$  production are shown to be worthless criteria. The sugars most frequently fermented by the various strains of *P. multocida* were dextrose, glucose, levulose, and saccharose. It is interesting to note that the majority of the strains fall into the Rosenbusch-Merchant Group II, i.e. they ferment xylose, but not arabinose and dulcitol. Group II was shown by them to represent strains from all animal species except birds, and it is intriguing to speculate on the apparent lack of avian strains in this summary.

The help given by Dr. Curt P. Richter and Dr. David Davis has been indispensable for this study. Also gratefully acknowledged are the valuable suggestions of Dr. Karl F. Meyer.



TABLE VIII

Bacteriological data from reports of human pasteurellosis. (A summary of published data from 1930-1947) See Table VII

REPORT	BIPOLARITY FIRST DETECTED			BIOCHEMICAL TESTS												ANIMAL PATHOGENICITY						AGGLUTINATION OF				SPECIAL REMARKS					
	Direct smear	Culture	After animal Passage	H <sub>2</sub> S Production	Growth on Potato	Dextrose	Glucose	Sucrose	Saccharose	Levulose	Maltose	Xylose	Galactose	Mannite	Arabinose	Dulcitol	Sorbitol	Mannose	Mouse	G-pig	Rabbit	Canary	Pigeon	Dog	Monkey		Patient-serum	Homologous Anti-serum	Control sera	Cross-agglutination with other <i>P. malleo-cida</i>	
1			+	+	0	A			A	A	A		A	A	-	-		A	+	+	+	0	0	0	0	1:200	+	-	+	First thought of H. influenzae	
2	+	+		0	0		A		A	A		A	A	A	-	-		A	+	0	0	0	0	0	0	0	0	0	0	0	First thought of H. influenzae
3			+	0	0														0	+	+	0	0	0	0	1:1000	0	0	0	0	First thought of H. influenzae
4	+	+		0	0	A			A	A	-			A		-		A	+	0	0	0	0	0	0	0	0	0	0	0	First thought of H. influenzae
5	+	+		0	0		A		A	A				A				A	+	0	+	+	0	0	0	0	+	0	0	0	First thought of H. influenzae
6		+		0	-		A		A	A				A		-			+	0	+	+	+	0	0	1:1600	0	0	0	0	First thought of H. influenzae
7		+		0	0	A			A	A		A		A					0	0	+	+	0	0	0	1:1600	0	0	0	0	First thought of H. influenzae
8	+	+		0	0		A		A	A		A		A		-			0	0	+	+	0	0	0	0	+	0	0	0	First thought of H. influenzae
9			+	0	0		A		A	A		A		A		-			+	+	+	0	0	0	0	-	+	0	0	0	First thought of H. influenzae
10	+	+		-	0		A		A	A		A		A		-		A	+	+	+	0	0	0	0	-	0	-	-	+	First thought of H. influenzae
11			+	0	0		A		A	A		A		A				+	+	0	0	0	0	0	0	0	0	0	0	0	First thought of H. influenzae
12	+	+	+	0	0	0	A	0	A	A		-		A		-		0	+	0	+	+	0	0	0	1/50	1:2400	0	1/320	0	First thought of H. influenzae
13	+	+		0	0	A	A	A	A	A				A				+	+	0	+	+	0	0	0	0	0	0	0	0	First thought of H. influenzae
14	+	+		0	0	A		A	A	A				A				+	+	0	+	+	0	0	0	-	0	0	0	0	First thought of H. influenzae

[illegible]

† = reaction positive

— = reaction negative

0 = reaction not mentioned

**A = acid without gas**

# BIBLIOGRAPHY

1. MIRICK, GEORGE S. ET AL. To be published.
2. MEYER, K. F. ET AL., J. Infect. Dis., 39: 386-412; 1926.
3. MOORE, U. S. Dept. of Agr., B.A.I. Bull No. 3, 1895.
4. SCHENK, Staatliche Bakteriologischer Untersuchungs-anstaltt, Munich, 1938.
5. KAPEL AND HOLM, Zentralbl. f. Chir., 57: 2906, Nov. 1930.
6. WEBER, Zentralbl. f. Chir., 68: 653, 1941. (Bacteriology described in Rimpau, München, Med. Wchnschr. 413: 1937.)
7. ALLIN A., Canad. M. A. J., 46: 48, 1942.
8. ALLOTT & AL., J. Path. & Bact., 56: 411, 1944.
9. HANSMANN & TULLY, Am. J. Clin. Path., 15: 312, 1945.
10. COOPER & MOORE, Lancet, p. 753, June 16, 1945.
11. RIVOALEN A., Bull. Soc. Path. Exot., 709: 1936.
12. BOISVERT & FOUSEK, J. A. M. A. 1902, 1941.
13. LENORMANT, Presse Med., 76-77, Sept. 3, 1941.
14. BAUMGARTEN, Lehrbuch der Path. Bakterien, 349, Hirzel, 1911.
15. HUTYRA, Kolle und Wassermann Handbuch, Vol. 6, part 1, 1925.
16. MANNINGER, Office Internat. des Epizooties, Budapest, 1934.
17. ROSENBUSCH AND MERCHANT, J. of Bact., 37: 69, 1939.
18. EMLER, J., Wildlife Management, Vol. 8, 3, July 1944.
19. MEYER, K. F. Personal Communication.
20. WEBSTER, J. Exp. Med., 51: 219, 1930.
21. GILTNER, L. T. Personal Communication.
22. RICHTER, CURT P. Personal Communication.
23. RICHTER, CURT P., J. A. M. A., 128: 324, June 2, 1945.
24. MULDER, J., Nederl. Tydschr. v. geneesk., 82: 977, 1938.
25. REGAMEY, Zentral-bl. f. Bakt., Abt I, 142: 431, Oct. 1938.
26. BROWN, J. HOWARD, Personal Communication.
27. The Infectious Diseases of Domestic Animals, William H. Hagan; Comstock Co. New York, 1943.
28. A System of Bacteriology, Vol. 4, Medical Research Council; London, 1929.
29. HUNDESHAGEN, K., Med. Klin. II, p. 1008, 1919.
30. Annals of the Pickett Thomson Research Laboratory; Monograph 16, Influenza, Balliere, Tindal & Cox; London, 1934.
31. CORONINI C., Wiener Med. Wochenschrift, 1043, 1937.
31. RIMBAUD & AL., Bull. et mem. Soc. med d. hop. de Paris, 305: 1931.
33. PLETTE, J. Nederl. Tydschr. v. Geneesk. 82: 6106, 1938.
34. LE CHUITON F. & AL., Compt. rend. Soc. de Biol., 130: 1096, 1939.
35. REINDERS & AL., Nederl. Tydschr. v. geneesk. Aug. 21, 1943.
36. SLECHT, H. Med. Klin., 27: 2, July 24, 1931.
37. LEVY-BRUHL, Rev. de Path. comparee., 34: 277, 1934.
38. FOERSTER, W. Klin. Wchnschr., 17: I, 599, 1938.
39. PELTIER & AL., Bull. Soc. Path. Exot., 31: 475, 1938.
40. PESTANA ET AL., Rev. Inst. Adolfo Lutz I, 357: Dec. 1941.
41. LUDLAM, G., J. Path. & Bact., 56: 307, 1944.
42. Queen and Quortrop, J. A. Vet. M. A., 108: 101, 1946.
43. Thjotta, Th. et al., Act. path. et microb. Scand. 23: 412, 1946.

## BOOK REVIEWS

*Actions of Radiations on Living Cells.* By D. E. LEA. Illus. 402 pp. \$4.50. Macmillan Company, New York, New York, 1947.

In this monograph, the author has given an excellent quantitative account of certain fundamental actions of x-rays and other ionizing radiations on living cells. The book begins with two chapters briefly discussing the physical properties and chemical effects of ionizing radiations, and then proceeds to discuss in considerable detail the target theory of radiation reaction and its application to problems in genetics and virus inactivation.

Within the chapters devoted to the genetic effects of radiation, a wealth of material has been presented on the structural changes produced by ionizing radiation in the chromosomes of *Drosophila melanogaster*,<sup>1</sup> *Tradescantia*, and other animal and plant forms. The frequency with which these changes occur has been considered in relation to the methods by which the several types of radiation have been applied. That many of the changes are caused by single ionizations has been convincingly presented.

The book seems of particular value because it brings together a mass of uncorrelated observations which have been made within the field of radiobiology over a period of years. The data have been given critical quantitative and statistical analysis by which the author seems to provide, beyond all reasonable doubt, a satisfactory explanation of the mechanism by which a small but important number of radiation reactions occur. The book should therefore constitute an excellent guide to those workers who may wish to apply ionizing radiations as tools in the investigation of many fundamental biological processes.

The book is well printed and it is of convenient size for ready reading. The graphical illustrations are clear and demonstrate satisfactorily the data which the author wishes to show. Certainly the book is a valuable contribution to the field of radiobiology.

R. H. M.

*Allergy in Theory and Practice.* By ROBERT A. COOKE. Illus. 572 pp. \$8.00. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.

This volume is a comprehensive presentation of the many scientific and clinical problems which the physician encounters in the field of allergy. In the foreword, the author states that "instruction in allergy has lagged in spite of its steadily increasing importance, as evidenced by its basic significance and broad applicability in the theory and practice of medicine and its various sub-divisions." The reviewer heartily endorses this statement. It is gratifying indeed to have in our libraries a book that so thoroughly and accurately covers this special field.

The work is a compilation of formal lectures which the author and the "New York Group," sponsored by the American College of Physicians, have presented so ably during the past few years to post-graduate students of allergy. With few

exceptions, the contributors have been students of the author and have worked under his direction for many years; naturally, his conservative judgment is reflected in the writings of these associates.

The lectures are divided into nine sections and an appendix. Within these nine sections, all fundamental aspects of allergy are discussed at length. Although there are differences of opinion concerning the mechanism of the "skin sensitizing antibody" and the "inhibiting antibody," the author gives an appraisal that is fair to each group of investigators.

Section I, devoted to the pathologic-anatomic aspects of allergy, emphasizes the role of allergy in disease. Although this section is brief, the discussion on the immuno-chemistry of allergens and antibodies is inclusive and instructive.

Sections II and III concern allergy of the respiratory tract. To an exceptional degree, this clinical discussion is concise and helpful. Unfortunately, the arrangement is not chronologic, for example, non-infective and infective asthma are discussed before hayfever and rhinitis are presented; yet the latter two conditions, in clinical practice no less important than the former, are often the fore-runners of the more serious complaints. The part relating to the differential diagnosis of bronchial asthma is condensed to five pages; thus, the information presented is little more than an outline. More importance should have been ascribed to this portion of the book, whether the publication is for the post-graduate student or for the under-graduate student.

Section IV, "Allergy of the Skin," is a compilation of lectures by the author, by Louis Schwartz, and by Dudley D. Stetson. Allergic dermatitis is so important in chemical industries, even in less hazardous occupations, that too much emphasis cannot be placed on this manifestation. These lectures adequately discuss the mechanism of allergic dermatitis, the occurrence of various types of dermatitis, and the treatment of the different clinical problems that are encountered.

Less understood allergic problems are discussed in Section V (Allergy of the Nervous System) and in Section VI (Allergy of the Cardiovascular System). The lectures in Section VII relate to other specialties: Allergy of the Digestive System; Allergy of the Eye; Allergy of Infancy and Childhood. The discussion of gastrointestinal allergy is conservative, and is in complete agreement with the experience of the reviewer. However, there are other physicians, also interested chiefly in allergy, who will consider this section too conservative, even inadequate.

In Section VIII there are a number of subjects which have been given special consideration by the author: for example, Bacterial Allergy in Relation to Diseases of Allergy; Inhalants; Foods and Drugs in Relation to Diseases of Allergy; and finally Physical Allergy. Many of the unusual reactions of these allergens are clearly explained in this series of lectures. Section IX should be especially helpful to the laboratory assistant. The discussion of skin testing, of preparation and standardization of extracts, and of laboratory procedures should prove particularly helpful to those physicians who contemplate the special field of allergy.

Page 148 contains a statement to which the reviewer must take exception. The author states that he does not concur in the belief that opium and its derivatives

should never be used in asthma. That many asthmatic patients have died following the injection of a small dose of morphine must be well known to the author and to other physicians with training in allergy. Death was due to sudden paralysis of a fatigued respiratory center in a patient who had been struggling for oxygen over a period of hours. Because such reactions cannot be foretold, I emphasize that the use of morphine in asthma is too dangerous a procedure.

In the appendix the author mentions, as if it were an afterthought, "a revival of interest in psychic and nervous factors in relation to disease—so-called psychosomatic medicine." Only two short paragraphs are devoted to this important subject, and the reviewer is given the impression that the author is not impressed with the importance of nervous factors in relation to allergic diseases. Of course, psychic factors cannot produce an allergic state, but pseudo-allergic states would not be diagnosed as "allergic" if specialists in the field of allergy were better psychiatrists. This is true especially in the study of patients suspected of an allergy to foods.

In general this is an excellent presentation, discussing and clarifying the theory, the clinical manifestations, and the treatment of allergic diseases. This volume will be most enlightening to all internists and to students who contemplate the practice of internal medicine.

L. G.

*Bacteriology, Laboratory Directions for Pharmacy Students.* 2nd Ed. By MILAN NOVAK and ESTHER MEYER. 247 pp. C. V. Mosby Company, St. Louis, 1947.

This manual of laboratory exercises for thirty-six periods is quite comprehensive and should provide an excellent course for either pharmacy or medical students. Methods, viewpoints and objectives are so diverse in bacteriology that every teacher in this field will find it easy to criticize a course outlined by others without implying criticism of the authors. Another factor which makes it difficult to publish a manual, which may be used to advantage in a number of schools, is the time factor. Not only the length of the course, number of exercises, but also the distribution of hours and days scheduled during the course may necessitate complete rearrangement of the material. Bacteria are living, growing organisms that do not skip idle days nor rest on Sunday. For this reason most teachers of bacteriology do and should write their own outlines. In doing so they frequently find valuable suggestions and material in such manuals as the one here reviewed.

J. H. B.

*Brown-Sequard, Charles-Edouard; A Nineteenth Century Neurologist and Endocrinologist.* By J. M. D. OLMSTEAD. 253 pp. \$3.00. The Johns Hopkins Press, Baltimore, Maryland, 1946.

This book comprises a recent course of lectures given at the Johns Hopkins Institute of the History of Medicine. It is a well-documented account of the life and achievements of one of the great nineteenth century physiologists. The author,

Dr. Olmstead, the Professor of Physiology at the University of California, is excellently fitted to evaluate the work of his fellow-physiologist. His appraisal of Brown-Sequard's scientific contributions forms the most valuable feature of the book. He shows well the extraordinary mixture of careful investigation and uncritical nonsense that characterized this "eccentric genius." The impetuous intuitive French approach to scientific investigation, and the frequent tendency to build sweeping generalizations on single observations are seen here, yet often the results are brilliant and the contributions lasting. There is some interesting discussion of the relative merits of pure and applied science, and also of the inter-relationship between medical research, teaching, and practice. Little attempt is made to portray the character of the man or the environmental forces that influenced his development. The biographical portion of the book is essentially factual, with the author's interpretive efforts being applied to the work of the scientist rather than to his personality. The book is attractively printed and well bound. It should be of interest to everyone interested in the development of neurophysiological thought during the last century.

G. G. M.

*Handbook of Commonly Used Drugs, A.* By MICHEL PIJOAN and CLARK H. YAEGER. 198 pp. \$3.75. Charles C. Thomas, Springfield, Illinois, 1947.

The authors have attempted to present, in highly abbreviated form, a résumé of current drug usage. The preface states that their purpose "is not to replace standard text-books of pharmacology, but to act as an adjunct and a guide in conditions where such texts are relatively unavailable." The presentation is somewhat on the model of Goodman and Gilman's "The Pharmacological Basis of Therapeutics." Thus each discussion of a drug is preceded by a short section on its structure and physiological effects. The discussion of the drugs as therapeutic agents is so brief as to cause confusion. This is particularly true in the chapter on sulfonamides. The toxicity of drugs is inadequately covered.

Chapter XVII contains some 45 pages (25 per cent of the text) on the treatment of tropical diseases. Many of these diseases have no specific treatment, so that the discussion of the disease is perforce limited to etiology, epidemiology and prevention. In the section on malaria, it should be pointed out that the suppressive regimen recommended for quinacrine (0.05 gm. daily and 0.1 gm. on Sundays) is lower than that found to be necessary by the Army. The suppressive regimen recommended for SN 7618 (0.3 gm. daily) is 5-6 times in excess, and approaches the toxic range.

The book is marred by an excessive number of errors of typography and spelling, so that it cannot be depended upon as a factual guide. One particularly serious mistake is that the metric hypodermic dosage of morphine is stated to be 0.515 grams. The structural formulas for neocinchophen, quinacrine, dilantin, homatropine, scopolamine, and other substances, are incorrect.

Because of dangerous brevity and the large number of errors, this book fails to fulfill its avowed purpose.

C. G. Z.

*If You Need an Operation.* Dr. RICHARD A. LEONARDO. 198 pp. \$3.00. Froben Press, New York, New York, 1947.

There is a great demand for popular books on medical subjects. Dr. Leonardo, whose facile pen has produced several rather successful books on medical history and philosophy, here turns his attention directly to the needs and worries of the laity. This book is a particularly useful one in its field and could be safely given to most intelligent patients. It answers their questions adequately and plainly and at the same time would not seem to implant new worries in their minds.

H. N. H.

*Jewish Luminaries in Medical History.* By HARRY FRIEDENWALD. 199 pp. \$3.00. Johns Hopkins Press, Baltimore, Maryland, 1946.

A scholarly little work for scholars. This lecture by Doctor Friedenwald, with a foreword by Henry Sigerist, is not merely pleasantly informative but is a key to the other monumental work, "The Jews In Medicine." It is a sine qua non for anyone interested in the history of medicine. The lecture and the list of titles serve alike to note some of the great Jewish figures in medicine and to emphasize the value of the continuity of their cultural thread.

C. R. A.

*Penicillin in Syphilis.* By JOSEPH EARLE MOORE. Illus. 319 pages. \$5.00. Charles C. Thomas, Springfield, Illinois, 1946.

In several ways this is a fascinating book. It discusses all of the general points relating to penicillin as far as were known at the date of publication, October 1946. It presents an accumulation of information, of clinical data, and of experimental work that has not been brought together elsewhere. And it is an outstanding example of the product of cooperative investigation.

This book records the work of 52 groups of clinics including four installations of the U. S. Army, one of the Navy, thirteen of the U. S. Public Health Service Rapid Treatment Centers, twenty-six civilian clinics, and eight laboratories of experimental syphilis. Never before has such widespread and intensive investigation been directed at any one therapeutic problem. The results are brilliant and satisfying. It should be pointed out that this cooperative method is possible only when established and uniformly organized clinics are available and when general interest and financial support center on one aspect of a clinical subject. This variety of cooperation was developed as a military measure during the last war. The technique of such studies should continue to be useful in certain instances during peacetime. One must not forget, however, that without the impetus of war, each clinic and laboratory will revert to its own research pattern in preference to a pattern submitted by a central agency.

Dr. Moore has an easy, refreshing, and sometimes dramatic style. The mass of material covered in this book is presented smoothly and interestingly. Even when the reader finds himself in slight disagreement with the conclusions that are put forth, he recognizes the thought behind the written word and appreciates that much that is discussed in this book is still in the process of being discovered.



There are something over 249 references to current literature. The index is excellent. This is a book that no one who is interested either in penicillin treatment of infectious diseases or in the treatment of syphilis can miss with impunity.

H. M. T.

*Rehabilitation Through Better Nutrition.* By TOM D. SPIES. Illus. 94 pp. \$4.00. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.

"The purpose of this monograph", Dr. Spies writes, "Is to call attention to the fact that we have shown that persons debilitated solely by nutritional deficiencies gain strength promptly following specific nutritive therapy." With this in view, Dr. Spies has summarized the experiences of the Nutritional Clinic at the Hillman Hospital, Birmingham, Alabama. Here, as elsewhere, nutritional deficiencies were observed not only in the indigent, but in those with erroneous dietary habits, food idiosyncracies or organic disease which interferes with the ingestion or utilization of food, in pregnant and lactating women and persons whose physical exertions have increased, and in persons chronically addicted to alcohol as well. Spies speaks of malnutrition as "primary" when it is the result of failure to eat an adequate diet, and "secondary" when the result of disease. He emphasizes that in human beings it is rare to see a naturally occurring deficiency of a single nutrient, a concept which we assume he limits to the adult age group. Instead he prefers to speak of "nutritive failure", a term describing a variable clinical picture as the result of the failure of one or more specific nutrients. Indeed, Dr. Spies believes that no two patients present the same clinical manifestations of deficiency. The rôle of adequate nutritional therapy in the rehabilitation of the malnourished was tested on 914 patients, selected because they were non-alcoholic adults, potentially capable of work, without serious organic disease, who had diagnostic evidence of one or more deficiency diseases and nutritive failure severe enough to prevent their working. These patients were treated in accordance with four therapeutic principles. Conditions causing excessive needs for the essential nutrients were relieved; co-existing disease was treated symptomatically; a liberal and well balanced diet was supplied, and the diet was supplemented with synthetic nutrients or concentrates. Treatment was not regarded as satisfactory until the patient had regained strength, his weight had returned to normal, and he was able to return to work and to continue to work regularly. Impressively, on this regime 893 patients were restored to gainful employment.

This manual was designed as a guide to therapeutics in nutritional failure for the use of medical students and physicians. With this in view, clinical descriptions of various deficiency states, illustrative case histories, and specific dietary and therapeutic regimens have been included. It is unfortunate that the brevity of the monograph has precluded greater detail in the description of the clinical pictures encountered, as well as the pathologic and experimental basis for the assignment of specific symptoms to specific deficiency states. Similarly the lack of a comprehensive bibliography limits the utility of this book to students of

nutritional disorders. These omissions lend the book a didactic air which does not jibe with Dr. Spies' own caution in the diagnosis of deficiency states. The monograph, however, is a useful exposition of the value of careful clinical analysis and aggressive therapy in the management of patients with nutritive failure.

O. D. R.

*Sex Education: A Guide for Parents, Teachers and Youth Leaders.* By CYRIL BIBBY. 311 pp. \$3.50. Emerson Books, Inc., New York, New York, 1946.

This book, written in recognition of the increasing need for a simple but comprehensive discussion of the subject matter of sex education, should prove to be a very helpful, practical guide for all those who are becoming more and more aware of the importance to provide our youth with well planned information on the topic.

The author devotes a large portion of the discussion to the integration of sex education with other subjects in school, while relegating the major responsibility for its teaching to persons outside the home. He does so with the intention of deemphasizing the special position sex has been given in the past and thus make it merely one aspect of one's biological development. The majority of parents, more unable than unwilling to assume the rôle of teaching, will welcome the school teacher's readiness to perform the difficult task for them, according to the author; discussions on the topic per se by itinerant lecturers should be limited to the exceptional occasion.

The book includes simply presented factual information on most all sexual topics while at the same time providing the instructor with the type of vocabulary most useful to convey the information to children; this is done by illustrative answers to questions which are likely to be submitted by them. With some justification, the author minimizes the position of the family physician in sex education, stressing his lack of educational ability and experience. On the other hand, it might be said about school teachers that they are by no means qualified to teach sex by virtue of their other educational experience. The author recognizes this point by advocating improvement in the curriculum of teachers' colleges.

All in all, the book is a courageous and successful attempt at clarification of an issue which, unfortunately, is still controversial. It should be read by everyone who has close contact with children and adolescents.

E. A.

*Surgeon's Domain, A.* By BERTRAM M. BERNHEIM. 253 pp. \$3.00. W. W. Norton and Company, Incorporated, New York, New York, 1947.

This book is an attempt to describe the life of a surgeon in everyday language for the lay reader. The success of Dr. Bernheim's other recent publications testifies to the popularity of such writing. The book contains many personal reminiscences of the early days at Hopkins.

H. N. H.

*Surgical Treatment of the Soft Tissues.* By F. W. BANCROFT AND G. H. HUMPHREYS. Illus. 520 pp. \$15.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1946.

This is a useful volume with chapters contributed by competent authors on a somewhat heterogeneous group of subjects. There is a sensible discussion of treatment of hernia and cryptorchidism. The results of treatment in the fields of plastic and maxillo-facial surgery are shown by pictures. There is a review of the physiologic alterations following burns, accompanied by description of a wide variety of local treatments, many of them now abandoned, and a voluminous bibliography. General and special infections of the soft tissues are taken up. There is a good discussion of chemotherapy, already somewhat out of date. Benign and malignant neoplasms of the breast and other soft tissues are satisfactorily covered. There is a valuable summary of the uses and limitations of x-ray therapy of neoplasms. An excellent essay on surgery of the large arteries by Herrmann and Reid is the highlight of the book. Here general principles and particular regions and problems are admirably clarified. The surgery of the veins and lymphatics is discussed helpfully.

It would be well in such compilations if the authors would be content with and agreed upon a definite limited audience. The reader who operates on aneurysms need hardly be told how to treat a lymphangitis or incise a paronychia. Parts of this book are too technical for the uninitiated, and others too elementary for the practicing surgeon.

W. E. G.

*Synopsis of Operative Surgery.* By H. E. MOBLEY. 2nd ed. Illus. 416 pp. \$6.00. The C. V. Mosby Company, St. Louis, Missouri, 1947.

This book presents all the standard operative procedures in a brief form, accompanied by suitable illustrations that give a clear understanding of the principle of the operation, but not elaborating on any of its details. It also tabulates the indications for the various procedures, but without discussion of the diagnosis or prognosis. It is not intended either as a textbook or system of operative surgery for the surgical specialist, but as a quick reference for those not too familiar with the various types of operation and interested more in the general scope of the operation than in its technical details. For this latter purpose it is excellent and should be useful to the returning veteran, the medical student, and those preparing for examination in surgical subjects.

R. T. S.

*Tuberculosis As It Comes And Goes.* By EDWARD W. HAYES, with Chapters by LAURENCE DE RYCKE. 2 Ed. 220 pp. \$3.75. Charles C. Thomas, Springfield, Illinois, 1947.

The second edition of "Tuberculosis As It Comes and Goes" brings to date valuable information for the instruction and understanding cooperation of the tuberculous patient with his doctor. Clearly and with accuracy the essential

facts are set down, and the only criticism of the text is that it assumes a familiarity with many terms that the average layman lacks. Perhaps this has a purpose—to have him seek such as a form of occupation and for emphasis! The chapters by Laurence de Rycke are done especially well and are to be commended to all who have any type of chronic incapacitation.

The book is entitled to a high place among those published for the guidance of all taking the "cure" for tuberculosis.

C. R. A.

*Uterotubal Insufflation.* By I. C. RUBIN. Illus. 453 pp. \$10.00. C. V. Mosby Company, St. Louis, Missouri, 1947.

In this well illustrated monograph of 453 pages Rubin has presented the subject of uterotubal insufflation and allied subjects as no one else could have done. No other gynecologist has had the extensive experience of the author, and no one has at his disposal such a great volume of carefully kept records. The monograph summarizes Rubin's experiences in his great contribution to gynecology. The material presented, however, is not limited to Rubin's but is also drawn from the literature. Nevertheless, it is a personal book, and, when one has read it, he cannot fail to know Rubin's views on the various phases of the subject.

Although a clinical point of view is maintained throughout, the first four chapters are concerned with the anatomy, pathology, and physiology of the fallopian tube.

The second part of the book covers the technique of tubal insufflation and diagnosis. It takes up in detail indications and contraindications for insufflation. The most favorable time for uterotubal insufflation is discussed, and the physical signs and symptoms associated with gas in the peritoneal cavity are described. The varied causes of tubal occlusion are also considered.

The third part of the book considers the therapeutic aspects of tubal insufflation for relief of sterility. Here, Rubin calls attention to the partially or lightly occluded tubes which he believes may be opened often by insufflation. He reports his own series of 3,200 cases, 590 of which subsequently became pregnant. It is noteworthy that 228 or 38.64 per cent of the 590 women became pregnant within two months after insufflation. Rubin also presents statistics from 65 other gynecologists, showing pregnancies occurring in 1.1 per cent to 39.0 per cent of the women upon whom the test was done.

In Chapter 11 Rubin discusses operations for restoration of tubal patency, particularly in their relation to tubal patency tests. He concludes that laparotomy for plastic tubal reconstruction is justified "where the patient is particularly anxious to become gravid and is willing to leave no stone unturned in the effort." He quotes results from a variety of authors. Probably the best cross section of results in this country is shown by Greenhill's report of questionnaires from most of the representative gynecologists. Only 4.4 per cent of the operations resulted in living babies. For comparison, Rubin calls attention to the therapeutic results of tubal insufflation in those cases in which tubal patency was improved after a

second and third insufflation. He had 438 patients falling into this category and 15.07 per cent of them became pregnant. It thus becomes apparent that surgery is never indicated until the therapeutic possibilities of repeated tubal insufflation have been exhausted.

The fourth part is concerned with a comparison of insufflation and lipiodol injection. Much space is devoted to relating the shortcomings and dangers of lipiodol injection. It appears to the reviewer that the author substantiates his objections to lipiodol injection with sound pathological and clinical data. The author also discusses the possibility of harm from insufflation with CO<sub>2</sub>. He justly concludes that "the untoward results from lipiodol injection outweigh in number and severity the accidents from CO<sub>2</sub> uterotubal insufflation, and that the latter are more avoidable, a circumstance which appears to be inherent in the nature of the medium."

A chapter is devoted to insufflation in relation to relief of dysmenorrhea. Although he quotes favorable results by Moench and Peterson, Rubin wisely states that "Final conclusions as to the therapeutic effect of tubal insufflation on dysmenorrhea cannot be drawn because they are as yet based upon too small a number of cases."

Finally, the results of most of the gynecologists of the country with tubal insufflation, as obtained by questionnaire, are recorded. If the answers to the questionnaire show nothing else, they at least demonstrate that tubal insufflation has become an accepted and much used procedure by practically all of the gynecological and obstetrical world.

The book can be used with profit by all gynecologists, obstetricians, and many general surgeons whose work includes the investigation and treatment of infertility.

R. W. T.

## NUTRITIONAL DERMATOSES IN THE RAT

### XII. THE INFLUENCE OF DEFICIENCIES ON THE EXTENT OF INJURY AND HEALING TIME OF LIQUID MUSTARD GAS BURNS<sup>1</sup>

MAURICE SULLIVAN

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The mechanism involved in the injurious action of mustard gas is not known. It is believed that its vesicant and necrotizing properties may result from a combination of the mustard gas molecule with cellular tissue which includes some protoplasmic protein vital to cell life and perhaps a portion of one of the enzyme systems (1). In order to collect data which may prove to be of value in the eventual elucidation of the biologic effects of mustard gas, it was considered desirable to investigate the effect of nutritional deficiencies on the extent and healing time of cutaneous injuries produced by the gas. The rat was chosen as the experimental animal for the investigation, as this species is easily susceptible to a miscellany of deficiencies manifested by cutaneous changes (2). By utilizing the experimental nutritional dermatoses of the rat, it was possible to evaluate the influence of single and combined deficiencies and to make comparative observations of the effects of chemical damage to normal skins and to skins variously altered by atrophy, hypertrophy, edema, hyperkeratosis, crusting, vascular dilatation and ulceration.

#### THE EFFECT OF LIQUID MUSTARD GAS ON NORMAL HUMAN SKIN

Contact with liquid mustard gas always results in vesication and necrosis unless the amount applied is extremely minute. Only a small per cent of the gas is absorbed by the skin. The greatest portion of the absorbed gas becomes fixed in the epidermis. The remainder, which is free, is transmitted via the circulation to other organs.

<sup>1</sup> From the Department of Medicine (Dermatology) and the Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University. Work Performed Under Contract OEM-CMR 82, Johns Hopkins University, Department of Medicine (Dermatology) for the Committee on the Treatment of Gas Casualties.

The cutaneous manifestations appear hours to days after contamination depending on the amount and state of the gas, the atmospheric conditions, and the sensitivity of the victim. Latent periods of two to six days have been noted after mild exposures (1). The extent of injury is always larger than the area contaminated. The peripheral area of erythema surrounding a central blister or central area of necrosis may have a diameter of one and a half to two times that of the skin area to which the vesicant was applied. Hypersensitivity to subsequent exposures frequently is established after cutaneous damage by mustard gas. Recrudescence, after apparent healing, sometimes occurs and is displayed as multiple vesicles on the periphery of the healing injury.

Davis (1) described the microscopic findings produced by mustard gas as follows: "There is a vacuolar or hydropic disintegration of the cells with eventual rupture of the cell membrane. Spaces formed by the progressive liquefaction of these cells become filled with fluid exudate to produce an intradermal vesicle. The base of the vesicle, which rests on the surface of the corium, presents an occasional remaining basal cell. The roof of the vesicle consists of an upper layer of epidermal cells that remain viable for a time and a lower layer of necrotic or disintegrating cells. The vesicle contains a homogeneous or fibrillar eosinophilic staining fluid in which there are a few polymorphonuclear leukocytes. Some of the hair follicles appear necrotic for a short distance from the surface, but there are no significant changes in the sebaceous glands, sweat ducts or sweat glands. There is some edema of the superficial corium with capillary dilatation and perivascular edema and mild perivascular infiltration of lymphocytes and polymorphonuclear cells." The description of Davis presumably applies to the conscious experimental contamination of the skin with small measured amounts of liquid mustard gas, as contamination with large amounts produces necrosis and ulceration of severe degree.

#### THE EFFECTS OF LIQUID MUSTARD GAS ON NORMAL RAT SKIN

Contact of liquid mustard gas with rat skin produces necrosis but never results in macroscopic vesiculation. The failure of mustard gas to react with gross vesiculation in rat skin, and in the skins of other small fur bearing animals, has been a subject of much speculation and

has prejudiced some investigators against the use of experimental animals for procedures calculated to measure the injurious cutaneous effects of the agent. The blistering or vesicant phenomenon is rigidly associated with the notion of "a vesicant", and considerable quantitative significance is attached by some observers to the sizes of experimentally produced vesicles and bullae. Such strict interpretation of quantitative blister significance does not hold when the gross effects and histopathology of mustard gas injuries are appreciated and broadly viewed. In the case of contamination with large amounts of liquid mustard gas vesiculation occurs only on the periphery of the injured area, the central portion of which is necrotic and dry. In such a circumstance, quantitative significance of blister area is obviously untenable. The daily and sometimes hourly increase in the size of vesicles and bullae due to the spreading effect through the skin and the recrudescence of vesicles in healing areas also preclude the strict quantitative value of vesiculation. Individual variations due to many factors, including hypersensitivity and increasing states of sensitivity, are additional arguments against the necessity for producing blisters when quantitative effect is the consideration. Failure to produce the manifestation of gross vesiculation in the rat was viewed as no disadvantage in this study, as it was found that it was possible to produce other measurable signs of cutaneous damage.

Fifteen hundred applications of Levenstein 95 per cent pure liquid mustard gas were made to the frontal, parietal and occipital regions of the scalp, the sides of the snout, the anterior and posterior surfaces of the ears, the necks, the shoulders, the backs, the flanks, the abdomens, the dorsal surfaces of the paws, the heels, the plantae, and the tails of 150 adult McCollum stock rats. Nembutal anesthesia was employed. The hair was shaved from the test areas with electric clippers immediately before each application. Small amounts of 180 to 350 gammas were applied with a cylindrical stainless steel rod having an applying surface of 6.5 mm. Larger amounts of 1 to 5 milligrams were applied with a specially constructed microsyringe. It was found that the most suitable sites for testing were the parietal regions of the scalp, the posterior aspect of the neck, the back, flank, and abdomen. In the anesthetized rats these locations are plane surfaces. They are also convenient locations for measurements and



observations. In these sites it was possible to afflict standard reproducible injuries which were remarkably uniform. The pattern of the injuries was similar for the different amounts applied. The extent of each injury was proportional to the amount of the vesicant applied. The glistening liquid remained on the surface for only a few seconds after the applications. Three to four hours later there was blanching in and immediately around the site of contamination. Twenty four to forty eight hours later there was a round or oval plaque in the center of which there was a pale area; as a rule the central area of paling constituted one-half of the plaque. In some cases the entire plaque was erythematous and edema extended to the periphery, resulting in a slightly raised erythematous plaque not unlike a flat topped or slightly convex urticarial wheal. In some of the severe reactions, there was a greater intensity of erythema; in a few instances punctate hemorrhage occurred. The first stage of erythema and/or edema lasted for 2 or 3 days. Serous exudate was then observed in the central portion within an erythematous halo that persisted for 5 or 6 days. Seven or 8 days after the application of mustard gas the halo of erythema subsided and was replaced by another halo which spread centrifugally and in which the surface product of normal sebaceous secretion was absent. The peripheral bands were noted particularly on the backs where a thin deposition of sebaceous material is present normally in adult rats. Crusting increased in thickness. When the crust was forcibly detached, there was an underlying superficial ulcer. The stage of crusting lasted 10 to 14 days, following which there was healing with superficial scar formation. During healing there was alopecia in the scarred area, but on the periphery of the scar there was often increased growth of hair. There was a gradual restitution of the formation and deposition of the brown sebaceous material, and the pale halo surrounding the scar was not evident 6 to 8 weeks after the injury. In pigmented areas the scars were depigmented.

For the purpose of making quantitative comparisons it was necessary to divide the visible signs into 2 categories, namely, those which could be conveniently measured in millimeters or centimeters and those which had qualitative significance only. In the first or "measurable" group were erythema, central pallor, ulceration, crusting,

failure of sebaceous secretion, and scarring, the diameters of all of which could be recorded. In the second, or "immeasurable" group, were such signs as intensity of erythema, punctate hemorrhage, depth and thickness of edema, crusts, ulcers, and infiltration. Although the latter group of signs contributed to the comparative qualitative evaluation of injuries, the former group of signs served principally as a basis for comparison in the deficient and control groups because it was possible to ascribe to them numerical values.

Microscopic examinations of skin biopsies taken 15 minutes, 30 minutes, 1 hour, 5 hours, 8 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 8 days, 15 days, 21 days, 22 days and 30 days after the applications revealed changes which accounted for the gross signs of injury. These changes were studied and recorded and compared later with the changes observed in the injuries of deficient rats and their normal controls. Fifteen minutes after application of liquid mustard gas there are no discernible changes in the epidermis. In the cutis there are mild diffuse edema, sparse cellular infiltration, and a few dilated and hyperemic blood vessels. At one hour some of the epidermal cells are shrunken and distorted. In the cutis there are edema, mild diffuse cellular infiltration and dilated hyperemic vessels. The walls of some of the vessels appear to be damaged. At 5 hours there is an increase in the number of distorted epidermal cells; a few are vacuolated. Throughout the cutis are diffuse cellular infiltration, edema and hyperemia. At 8 hours the cellular infiltration extends deeper into the cutis. At 24 hours there are numerous distorted, flattened, vacuolated cells in the epidermis. There is acantholysis of the basal layer, and immediately beneath the epidermis are patchy areas of edema. The cells of the sebaceous glands and hair follicles are distorted; some are shrunken and some are dilated and disintegrated. There is an increasing amount of edema in the lower half of the cutis. At 48 hours there are several well defined areas of vesiculation in the epidermis, although grossly vesiculation is undetectable. Numerous areas of the epidermis are invaded by exudative cells and the structure of the epidermis is no longer recognizable. Many of the hair follicles and sebaceous glands are infiltrated by cells. In the cutis there is still diffuse cellular infiltration, hyperemia and edema. At 3 days there is separation of the epidermis from the

cutis and crusting on the surface. Some of the sebaceous glands are still intact but the majority have been destroyed. There is some edema and cellular infiltration throughout the cutis, most extensive in the lower half. The connective tissue shows some fragmentation. The underlying muscle is undisturbed. At 8 days a thick crust replaces the destroyed epidermis. Throughout the cutis there is extensive cellular infiltration which is most prominent in the perifollicular regions. In the lower cutis there are dilated hyperemic vessels and edema. At 30 days there is restitution of the epidermis, but in the injured area the epidermal cells are flattened and distorted, and there is destruction of the hair follicles in the central area. New connective tissue occupies the upper portion of the cutis.

When liquid mustard is applied to or accidentally comes into contact with human skin hypersensitivity to subsequent exposures frequently occurs. It was important to determine whether a state of hypersensitivity could be produced in rats and to know whether hypersensitivity and varying degrees of sensitivity should be taken into consideration in evaluating results of mustard gas injuries in rats.

Prior to the report of Kenton (3) in 1941, the rat was considered an unsuitable animal in which to produce phenomena of hypersensitivity. Numerous attempts to sensitize rats to egg white have failed (4). Several investigators have recorded their failures to sensitize rats to other substances (5). Kenton (3) showed that the rat responded to repeated antigenic stimuli with only slight antibody production and that it was not possible to demonstrate the Arthus phenomenon in the skin of actively immunized rats. However, when large amounts of a potent immune serum were administered to rats by passive transfer the Arthus phenomenon occurred regularly upon injection of the specific antigen. According to Kenton, these observations indicated that "the tissue of the rat will respond with typical inflammatory reaction whenever antigen and antibody are present in sufficient concentration".

Experiments 1 and 2, described below, demonstrate that: a. It is possible to produce in rats a phenomenon of hypersensitivity to liquid mustard gas by a method of multiple applications in which mustard gas is reapplied to sites previously injured by mustard gas. b. The phenomenon of hypersensitization may be avoided if each of

the multiple applications is made on a skin site previously undamaged by mustard gas.

*Experiment 1:* Thirty-three adult McCollum stock rats were divided into group A and group B. During the 5 months' period of the experiment both groups were reared under identical environmental and dietary conditions.

To the 14 rats in group A applications were made in a manner calculated to produce hypersensitization. With a rod having an applying diameter of 6.25 mm., 3 equidistant applications were made on the right side of the back of each rat. Approximately 1 week later 3 similar equidistant applications were made on the left side of the back of each rat. Again, approximately 1 week later, 3 similar equidistant applications were made on the mid line of the back of each rat. After stages of erythema, edema, ulceration and crust formation, each injured area healed by scarring. At 3 weeks, 5 weeks and 7 weeks after the initial applications, mustard gas was reapplied respectively to one of the 3 linear groups of 3 scars. At each of the previously injured areas where the mustard gas was reapplied, there was repetition of the stages of injury previously observed, and eventually there was healing with more extensive scar formation than that which followed the initial injuries. On 15 occasions an interesting phenomenon was observed 24 hours after the reapplication of mustard gas to 3 previously injured sites (scars) in one row. In and circumjacent to the 3 scars in one of the other rows erythema and edema were remarked. The signs of reactivation subsided in 24 hours whereas the signs of inflammation at the sites of reapplication persisted and produced ulceration and scarring. Reactivation in the scarred areas was thus accomplished by reapplying mustard gas to other previously injured (scarred) sites. Twelve weeks after the initial applications of mustard gas, single applications were made with the same rod at sites that were not previously injured. There was no evidence of reactivation in the previously injured areas. The extent of the injury was equal to that which followed the initial applications.

*Experiment 2:* To each of the 19 rats in group B applications were made with 8 smaller, graded applicators. On the backs and abdomens 3 rows of 7 applications were made with applicators having diameters of 0.5, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5 mm. Reapplications were made in the manner described above (group A). The same phenomenon of reactivation was observed. Two months later numerous applications were made with a rod having a 6.25 mm. diameter to *previously uninjured* sites on the head, ears, neck, flanks, paws, and tail. Over a period of 4 months many such applications were made to uninjured areas and the previously injured (scarred) sites were carefully avoided. There was no evidence of the reactivation phenomenon in the 4 months' period of observation. During the 4 months' period the average extent of injury at every site equalled the average extent of injury observed in rats to which only single applications had been made.

In all subsequent experiments care was exercised to avoid the application of the

vesicant to previously injured sites and the consideration of individual hypersensitivity was disregarded in evaluating results.

Observations of the extent of injury and healing time were made in groups of rats conditioned by deficiencies of carbohydrate, protein, fat, riboflavin, pyridoxine, pantothenic acid, biotin, vitamin A, and magnesium, and deficiency of the vitamin B complex other than thiamine. Cystine and choline, para-amino benzamide and large doses of vitamin B complex were administered to rats to determine the influence of these agents on healing time. Following are summaries of the experiments:

#### CARBOHYDRATE DEFICIENCY

*Experiment 3:* A litter of 8 matched young rats was divided into 2 groups, A and B. To the control group A, the following diet was fed: sugar 54%, casein 20%, lard 10%, salts 6%, yeast 10%. The sugar content of group B was reduced from 54% to 24% and the percentages of casein and lard were increased so that the diet was composed of 24% sugar, 30% casein, 30% lard, 6% salts, and 10% yeast. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E to both groups. The growth curves in the 2 groups were approximately equal during the next 3 weeks. A dose of 1.77 milligrams of liquid mustard gas was applied to the shaved back of each rat. There were no general ill effects. The rats were observed for 2 months and comparative measurements were made weekly. Rate of healing and extent of injury were approximately the same in each group.

*Experiment 4:* A litter of matched young rats was divided into 2 groups, C, consisting of 2 rats, and D, consisting of 6 rats. To the control group C, the following diet was fed: sugar 54%, casein 20%, lard 10%, salts 6%. The sugar content of the diet fed to group D was decreased from 54% to 24% and instead of increasing the percentages of casein and lard to make up the deficit, 30% agar-agar was added so that the ration consisted of 24% sugar, 20% casein, 10% lard, 6% salts, 10% yeast, and 30% agar-agar. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E to each group. The weight increase in group D was considerably less than that in group C. The control rats weighed 106 and 132 grams and the deficient rats varied from 50 to 95 grams. To the shaved backs of the control rats, and to 4 of the rats in group D, doses of 1.77 milligrams of liquid mustard gas were applied. Two of the deficient rats died 5 days after the applications. The other 2 survived for 2 months and continued to gain weight comparable to the 2 deficient litter mates that were not burned. Leukocyte counts were made before each application and 3 days later. The leucopenia which followed was not more marked in the deficient rats than in the control rats. In the deficient rats that were burned, there developed transient signs of pantothenic acid deficiency and

abdominal distention. Healing time and extent of injury were approximately equal in each group.

*Conclusion:* Carbohydrate deficiency apparently had no influence on healing time and extent of injury due to liquid mustard gas, when the deficiency of carbohydrate in the diet was compensated for by an increase in protein and fat. When the deficit of carbohydrate in the diet was not replaced by protein and fat, and inanition resulted, there were signs of transient pantothenic acid deficiency in the burned rats, although the cutaneous signs of injury were not enhanced and healing was not prolonged.

#### SEVERE PROTEIN DEFICIENCY

*Experiment 5:* A litter of matched young rats was divided into group A, consisting of 2 rats, and group B, consisting of 4 rats. To the control group A the following diet was fed: casein 20%, lard 10%, sugar 54%, salts 6%, and yeast 10%. A low protein ration was fed to the rats in group B; the percentage of casein was reduced from 20% to 8%, and the percentages of lard and sugar were increased to 16% and 60% respectively. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. During the first 2 weeks after the diet was started the weight gain in group B was less than that in group A. The doses of liquid mustard gas were calculated on a weight basis. To the shaved back of each rat 59 milligrams per 20 grams of body weight was applied with a microsyringe. The dose for the control group was 2.36 milligrams and that for the deficient group was 1.77 milligrams. Despite the fact that smaller doses of liquid mustard gas were applied to the deficient rats the extent of the injuries was greater and healing was delayed for three weeks (Figure 1).

The stage of deficiency in Experiment 5 was advanced and one not likely to be encountered in humans. Therefore, another experiment was performed in which adult rats were used in order to produce a less depleted condition in the animals.

#### PROTEIN DEFICIENCY OF MILD TO MODERATE DEGREE

*Experiment 6:* Forty six adult rats ranging in body weight from 195 to 235 grams were divided into 2 groups, C and D. To the control group C consisting of 12 animals was fed the following diet: casein 20%, sugar 54%, lard 10%, yeast 10%, salts 6%. Oleum percomorphum and alpha tocopherol supplied vitamins A, D and E. To the deficient group D was fed the same diet which had been used in Experiment 5 to produce the severe state of deficiency in the young rats. The rats in group D gained weight at a less rapid rate than the rats in the control group.

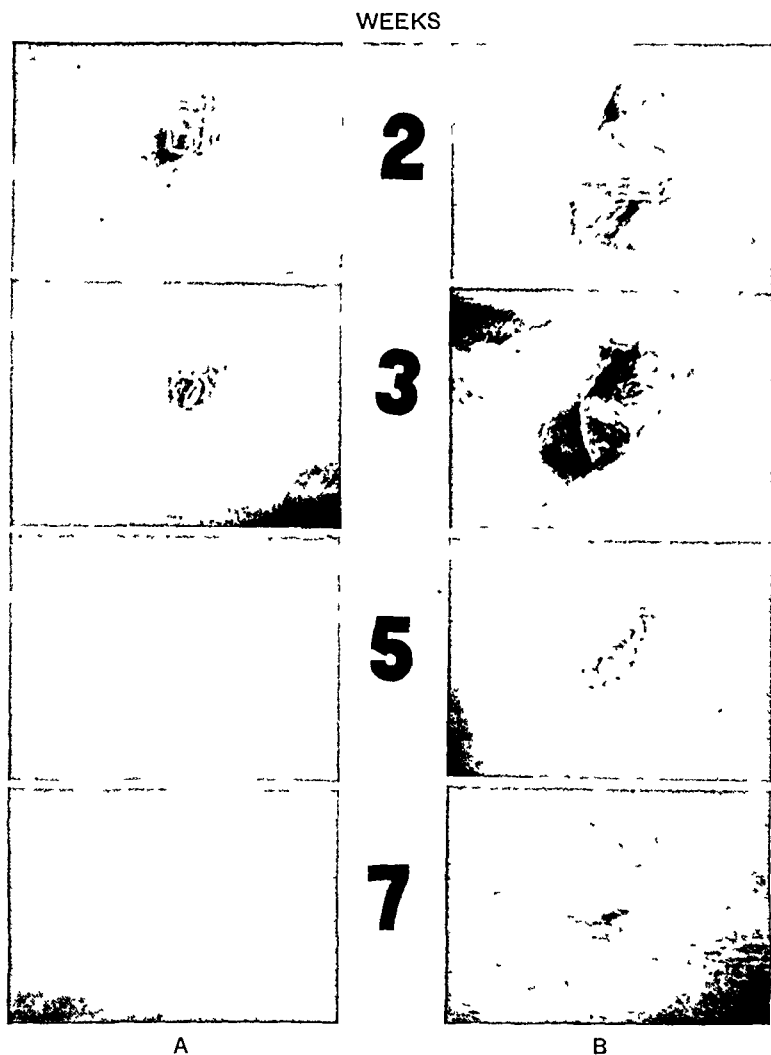


FIG. 1. (Experiment 5.) The serial stages of injury and healing in severe protein deficiency are portrayed at 2, 3, 5, and 7 weeks in the column B. In the control group, column A, left, the extent of injury was less than in the deficient group, column B, right. In the control group the injuries healed in 4 to 5 weeks. In the deficient group healing was not complete at 7 weeks.

C. However, the differences in the weight curves were only slight in contrast to the striking differences in the weight curves in Experiment 5 with young rats. Adult rats may be maintained on this relatively low protein diet (8% casein) for several months whereas the same diet will not support life in recently weaned rats. Between the fourth and fifth weeks after the diet was started, 1.77 mg. of liquid mustard gas was applied to the shaved back of each of 25 rats from group D. Nine rats in group D were not burned in order that they might serve as "deficient controls." All the rats survived. There were no general ill effects after the applications of mustard gas to the deficient rats. The rats in group D continued to gain weight for 2 or 3 weeks after the application of mustard gas. Thereafter the weights were usually stationary. A comparison of the weight curves of the 25 deficient rats that had been burned, and the 9 deficient rats that had not been burned showed no differences. All the deficient rats showed signs of deficiency 8 to 10 weeks after the beginning of the experiment. They were weak; in the majority the fur was unkempt and lacked luster. Animals from each group were sacrificed one week after the application of mustard gas. Microscopic examination of sections from the injured skin and of sections from the spleen, liver and bone marrow disclosed no findings indicating distinctive or characteristic tissue changes in the two groups. Leukocyte counts before and after the application of the gas showed no distinctive differences in the deficient (D) and control (C) groups. Weekly comparisons of injuries in the two groups showed that the extent of injury was approximately equal and that healing occurred with no delay in the deficient group.

*Conclusion:* The findings in Experiments 5 and 6 indicate that in protein deficiency of an advanced degree the extent of injury due to mustard gas is enhanced and healing time is retarded. Protein deficiency of a mild to moderate degree apparently has no effect on extent of injury and healing time.

#### FAT DEFICIENCY

*Experiment 7:* To a group of 30 young rats the following diet was fed: casein 64%, sugar 20%, salts 6%. Into each kilogram of the diet was incorporated: thiamine 20 milligrams, riboflavin 10 milligrams, nicotinic acid 20 milligrams, pantothenic acid 15 milligrams, pyridoxine 10 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams, and cystine 500 milligrams. Vitamin A and vitamin D were supplied by carotene and calciferol and each rat received 10 milligrams of alpha tocopherol per week to furnish vitamin E. During the first 5 months of the experiment there was a gain in weight. After the sixth month the weight was more or less stationary and the rats weighed approximately 120 to 130 grams; there was generalized scaling which was not preceded by or accompanied with erythema or edema; there was loss of elasticity and atrophy of the skin. These are the classical cutaneous signs of fat deficiency in the



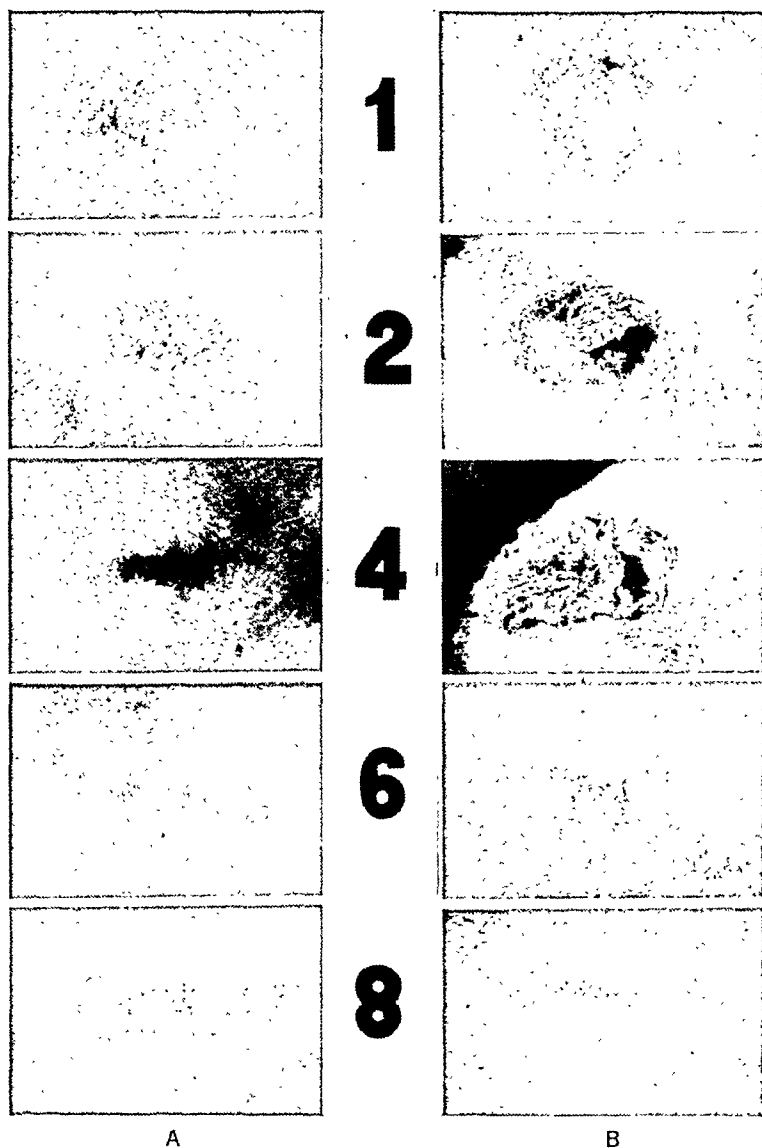
rat. Rats of comparable weights were selected for controls, inasmuch as litter mates of the same age would have weighed twice as much. By the rod method small quantities, 180 to 352 micrograms, of liquid mustard gas were applied to the shaved backs of the fat deficient and normal rats. There were no manifestations of general ill effects similar to those observed after the application of larger quantities of liquid mustard gas. Numerous comparative measurements were made, and the results showed that the extent of injury was greater in the deficient rats and that the healing was delayed for 2 to 4 weeks (Figure 2). The scars in the skin of the deficient rats were atrophic and were traversed by telangiectases. Histologic examination revealed no characteristic differences except that the damage was more extensive in the fat deficient rats.

*Conclusion:* Healing was delayed and extent of injury due to mustard gas was greater in fat deficient than in normal rats.

#### RIBOFLAVIN DEFICIENCY

*Experiment 8:* A litter of 10 young rats was divided into 2 groups A, and B. The control group A consisted of 2 animals, and the deficient group B consisted of 8 animals. To the control group A, the following diet was fed: casein 20%, sugar 54%, lard 10%, and salts 6%. Five hundred milligrams of cystine were added to each kilogram of the diet. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. Into each kilogram of the ration the vitamin B complex was incorporated as crystalline supplements in the following amounts: thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, pantothenic acid 15 milligrams, nicotinic acid 30 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams. To the deficient group B was fed a diet similar to that of group A with the exception that the supplement of riboflavin was omitted. In the ensuing month there was a well sustained increase of weight in the control group A. In the deficient group B weight was either stationary or very slightly increased. The rats in group B were humped and weak. Their furs were disheveled and infested with pediculi; these are the classical signs of riboflavin deficiency (6). To the shaved backs of both groups liquid mustard gas was applied. Inasmuch as the deficient rats weighed considerably less than the control rats, the dose was calculated on a weight basis, namely 0.59 milligrams per 25 grams of body weight. The dose administered to the control rats was 1.77 milligrams. The dose administered to the deficient rats was 1.18 milligrams. Three of the rats in the deficient group B died 1 week after liquid mustard gas was applied. The others survived for 6 weeks when they were sacrificed. In spite of the fact that smaller doses of liquid mustard gas were applied to the backs of the deficient group the extent of injury in each case was larger than that in the control group, and healing was delayed 2 to 3 weeks (Figure 3). Erythrocyte and leukocyte counts before and after showed no characteristic differences for the 2 groups. Examination of the spleen and bone marrow disclosed no striking differences in the control and deficient groups.

## WEEKS



A

B

FIG. 2. (Experiment 7.) In column A, left, the serial stages of injury in a normal rat at 1, 2, 4, 6, and 8 weeks are portrayed. A comparison of the photographs in column B, right, illustrating the injury in a fat deficient rat, shows that the extent of damage is greater in the deficient rat. There was less infiltration in the normal rat's skin than in the deficient rat's skin, although this is not well illustrated in the photographs. Three to 4 weeks after the application of mustard gas to normal rats there was healing with scar formation; in the fat deficient rats crusting and ulceration were extensive at this stage. In 6 to 7 weeks after the injuries, scar formation replaced the eschars in the fat deficient rats, and in 8 weeks healing was complete. Note the delay of healing in the fat deficient rat.

*Conclusion:* Healing was delayed and the extent of injury was greater in riboflavin deficient rats than in normal rats.

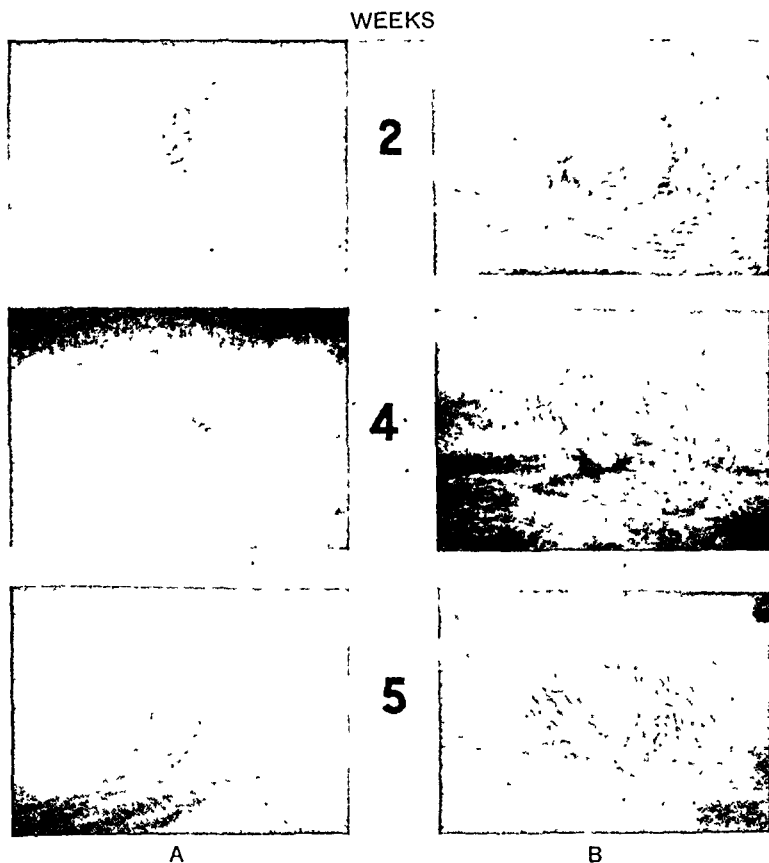


FIG. 3. (Experiment 8.) In column A, left, are portrayed at 2, 4, and 5 weeks the serial stages of injury produced by the application of 1.77 milligrams of liquid mustard gas to a normal rat. In column B, right, are portrayed at 2, 4, and 5 weeks the serial stages of injury produced by the application of 1.18 milligrams of liquid mustard gas to a riboflavin deficient rat. Healing was delayed in the deficient rat and the extent of injury in the deficient rat was much greater than in the normal rats.

#### PYRIDOXINE DEFICIENCY

*Experiment 9:* A litter of 10 young rats was divided into group A, consisting of 4 rats, and group B, consisting of 6 rats. To the control group A was fed the fol-

lowing diet: casein 20%, sugar 54%, lard 10%, and salts 6%. Five hundred milligrams of cystine were added to each kilogram of the ration. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. The vitamin B complex was supplied as crystalline supplements and was incorporated into each kilogram of the diet in the following amounts: Thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, nicotinic acid 30 milligrams, pantothenic acid 15 milligrams, choline chloride 1000 milligrams, inositol 375 milligrams, and para-amino benzoic acid 375 milligrams. The diet of the deficient group B was similar except that pyridoxine was omitted. In the ensuing 2 months, there was a well sustained increase of weight in the control group A. In the deficient group B there was a slight increase in weight, and signs of pyridoxine deficiency were manifested by the peripheral symmetric dermatitis which characterizes the disease (7). Inasmuch as the rats in the control group weighed approximately twice as much as those in the deficient group, the dose of mustard gas was calculated on a weight basis. Fifty-nine hundredths milligram of mustard gas per 25 grams of body weight was applied to the shaved back of each rat. The dose administered to the control rats was 2.36 milligrams. The dose administered to the deficient rats was 1.18 milligrams. In the deficient rats, the extent of injury was approximately the same or slightly larger than that of the control animals. However, it should be pointed out that the dose was smaller, so that a smaller lesion should have resulted. Healing was delayed 2 to 3 weeks in the deficient group (Figure 4). There were no systemic ill effects in the deficient group. Erythrocyte and leukocyte determination before and after showed no essential differences in the control and deficient groups. Examinations of the bone marrow and of the spleen showed no striking differences in the control and deficient groups.

*Conclusion:* Healing was delayed and the extent of cutaneous injury was slightly greater in pyridoxine deficient rats than in normal rats.

#### PANTOTHENIC ACID DEFICIENCY

*Experiment 10:* Twenty young rats were divided into 2 groups, A and B. To the control group B the following diet was fed: casein 20%, sugar 54%, lard 10%, and salts 6%. Five hundred milligrams of cystine were added to each kilogram of the diet. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. Into each kilogram of the diet the vitamin B complex was incorporated as crystalline supplements in the following amounts: thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, pantothenic acid 15 milligrams, nicotinic acid 30 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams. To the deficient group B consisting of 16 rats was fed a similar diet with the exception that the supplement of pantothenic acid was omitted. In the ensuing 2 months there was a well-sustained increase in weight in the control group A. In the deficient group B there was only a slight increase in weight. Extensive alopecia, dermatitis and "rusting" occurred in the albinos,

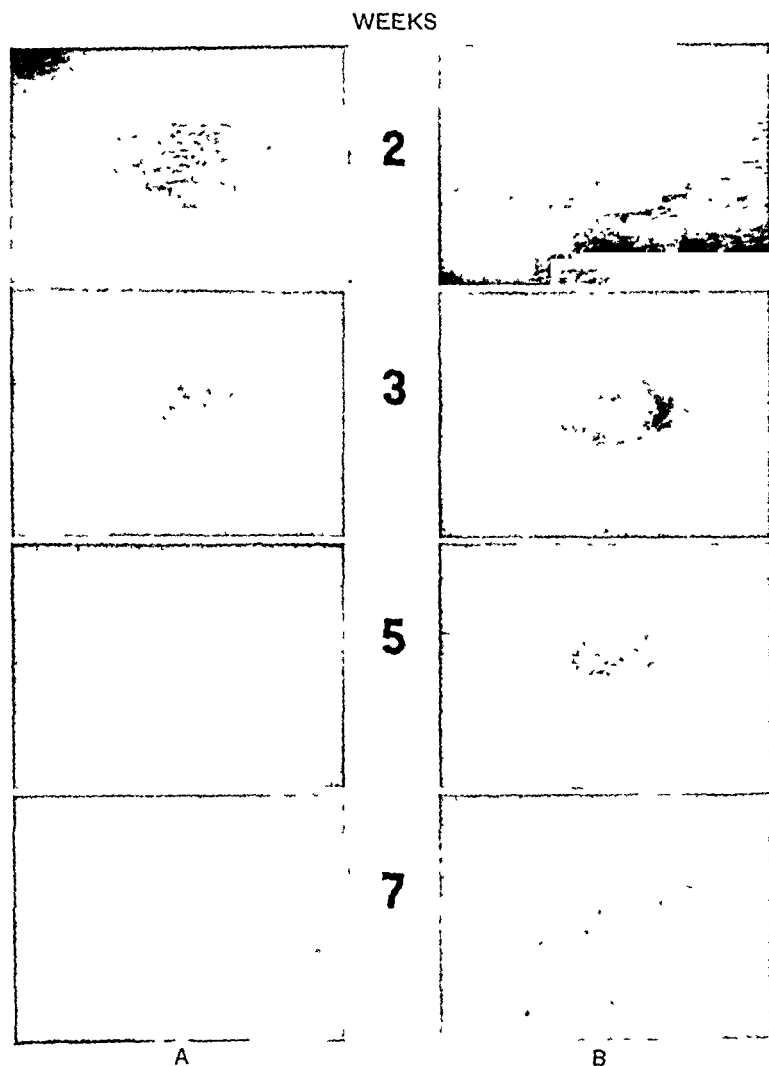


FIG. 4. (Experiment 9.) In column A on the left are portrayed at 2, 3, 5, and 7 weeks stages of injury produced by the application of 2.36 milligrams of mustard gas to a normal rat. In column B on the right are portrayed at 2, 3, 5, and 7 weeks the serial stages of injury produced by the application of 1.18 milligrams of mustard gas to a pyridoxine deficient rat. Healing was delayed for 2 to 3 weeks in the deficient rat and the extent of injury (column B) was slightly greater than that in the normal rat (column A).

and symmetric pattern achromotrichia was observed in the hooded rats; these are the classical signs of pantothenic acid deficiency (8). Because the control rats weighed 2 to 3 times as much as the deficient rats, the doses of mustard gas were calculated on a weight basis. Fifty-nine hundredths milligram per 20 grams of body weight was administered. One and eighteen hundredths milligrams and 1.77 milligrams were applied to the deficient rats, and 2.95 milligrams were applied to the rats of control group A. Weekly measurements showed that injuries which were produced when doses of 1.18 milligrams and 1.77 milligrams of mustard gas were applied to the pantothenic acid deficient rats were slightly larger than the injuries produced by doses of 2.95 milligrams of mustard gas which were applied to the normal rats. Healing time was approximately the same despite the larger dose administered to the control group. Microscopic examination of the bone marrow, spleen and skin of the deficient rats showed no distinguishing features when compared with sections of the control rats. Leukocyte counts before and 3 days after application of mustard gas showed no characteristic differences for the 2 groups.

*Experiment 11:* A litter of 8 young rats was divided into groups C and D. The control group C consisted of 2 rats, and the deficient group D consisted of 6 rats. To the control group C the diet described in Experiment 10 was fed. To the deficient group D the deficient diet described in Experiment 10 was fed. Two months later doses of 1.77 milligrams of mustard gas were applied to the deficient and control rats in order to evaluate the effect of the same dose in the 2 groups, rather than a dose calculated on a weight basis as in Experiment 10. Weekly measurements showed that the injuries in the deficient rats were greater than those in the control rats, and that healing was delayed for 1 to 2 weeks. Leukocyte counts before and 3 days after showed no characteristic differences in control and deficient groups. Microscopic examination of bone marrow, spleen and skin of deficient rats showed no distinguishing features when compared with sections of the control rats.

*Experiment 12:* Three young rats that had been depleted of pantothenic acid for 2 months and that manifested the characteristic signs of pantothenic acid deficiency were burned with 1.77 milligrams of mustard gas. Immediately after they recovered from the nembutal anaesthesia administered just before the application of mustard gas, supplements of pantothenic acid were incorporated into the previously deficient diet. During the ensuing weeks signs of the deficiency disappeared and the rats rapidly gained weight. Weekly measurements of the injuries showed that there was an acceleration of healing when compared with the injuries produced by an equal amount administered to the deficient rats in Experiment 11, and the rate of healing was approximately equal to that of the control group, C, in Experiment 11. Blood counts before and after showed no characteristic differences when compared with the leukocyte counts made before and after the application of mustard gas to the untreated animals of Experiment 11.

*Conclusion:* The findings in Experiments 10, 11, and 12 indicate that pantothenic acid deficiency is conducive to the production of

larger mustard gas injuries. A comparison of the results of these experiments with similar experiments, in which the influence of deficiencies of riboflavin and pyridoxine were studied, shows that the effect of pantothenic acid deficiency on the mustard gas injury was less deleterious than that of riboflavin deficiency or pyridoxine deficiency.

#### BIOTIN DEFICIENCY

*Experiment 13:* Biotin deficiency signs were produced in 20 recently weaned rats by feeding a diet composed of cornstarch 177 grams, sucrose 185 grams, dried uncooked commercial eggwhite 300 grams, McCollum salts (No. 51) 58 grams, peanut oil 150 grams. Vitamins A, D, and E were supplied by oleum percomorphum and alpha tocopherol, and thrice weekly each rat received a supplement of thiamin 50 gammas, pyridoxine 50 gammas, pantothenic acid 100 gammas, riboflavine 40 gammas and nicotinic acid 30 gammas. The rats were weak, humped and afflicted with generalized pruritic exfoliative dermatitis. A thick dark brown material consisting of crusts, scales, sebum, broken off hair shafts covered their skin in the fully developed stage of the disease. Doses of 180 to 350 gammas of liquid mustard gas were applied. The thick brown coating appeared to offer protection against the gas as the resulting burns were slight. The brown coating was scraped from the backs of the rats and spread onto the caudal portions of normal rats. Liquid mustard gas was applied to the cephalad portions of the normal rats, as well as to the caudal portion, which were coated with the brown material from the biotin deficient animals. The coating in this circumstance afforded no protection. A mild degree of biotin deficiency was then produced in 16 adult rats. Doses of 1.77 milligrams of mustard were applied. The extent of injury was slightly larger and healing was slightly delayed when compared with injuries in normal litter mates.

*Conclusion:* Some degree of protection against small doses (180 to 350 gammas) of liquid mustard gas apparently was afforded by the thick coating of scales, crusts, fat, and hair on biotin deficient rats. However, when larger doses (1.77 milligrams) were applied to adult biotin deficient rats in which the thick coating was not present, the extent of injury and healing time were greater than in normal rats. The significance of these findings is not apparent. (See discussion.)

#### VITAMIN A DEFICIENCY

*Experiment 14:* After a preliminary unsatisfactory experiment with acute vitamin deficiency in recently weaned rats, it was decided to produce chronic vitamin A deficiency in older rats for this experiment, as the survival period in chronic vitamin A deficiency in adult rats is sufficiently long to make observations over

periods of 2 months or more. Therefore, 8 three months old rats were selected and divided into 2 groups, A and B. To the control group A the following diet was fed: casein 18%, sugar 56%, lard 10%, salts 6%, yeast 10%. Each rat received supplements of oleum percomorphum equivalent to 2800 international units of vitamin A and 400 international units of vitamin D. To the deficient group B was fed the same diet with the exception that the supplement of oleum percomorphum was omitted and vitamin D was supplied as viosterol. During the ensuing 3 months weight gain was approximately equal in the 2 groups. During the fourth month the rats in the deficient group lost weight and ocular and nasal signs of vitamin A deficiency were apparent.

To the shaved back of each rat 1.77 milligrams of mustard gas were applied. Weekly measurements for the next 8 weeks showed no difference in the extent of the injury or healing time of the 2 groups. Microscopic examination of the injured areas of skin showed no distinctive or unusual features in the deficient rats. Leukocyte counts before and three days after the application of mustard gas showed no characteristic differences in the two groups.

*Conclusion:* The extent of injury due to liquid mustard gas and healing were not affected by vitamin A deficiency in adult rats.

#### DEFICIENCY OF THE ENTIRE VITAMIN B COMPLEX OTHER THAN THIAMINE

*Experiment 15:* To 9 rats, each weighing approximately 30 grams and 21 days old, the following diet was fed: casein acid washed 18%, sucrose 66%, butter fat washed 8%, McCollum salts, No. 51 6%, cod liver oil 2%. A supplement of 2 milligrams of thiamine was incorporated into each kilogram of ration. Six control rats were fed the above diet plus 10 per cent Anheuser Busch yeast. In 6 weeks the rats receiving the experimental ration were weak, atrophic and humped. The skin and its appendages were atrophic. Applications of mustard gas were made on the mid parietal regions with stainless steel rods having applicating diameters of 6.25 millimeters. In the 8 control animals, the reaction patterns corresponded to those observed in numerous other normal rats. One day after the application there was noted a localized round or slightly oval area of erythema 10 to 14 millimeters in diameter, in the center of which there was a 6 to 8 millimeter pale area. Two days later there was evidence of exudation in the central portion and peripheral extension of the erythema for a distance of 2 to 4 millimeters. In three to four days there was evidence of exudation in the central portion and peripheral extension of the erythema for a distance of 2 to 4 millimeters. In three to four days there was an increase in the amount of exudation with the formation of a dark brown rather circumscribed crust which was adherent to the skin for 10 to 15 days. The crust was then easily detached or spontaneously came loose. Underlying the crust was a superficial scar of 10 to 15 millimeters in diameter. In 2 or 3 weeks the scars contracted to 5 or 6 millimeters. During all stages of the reaction the extent



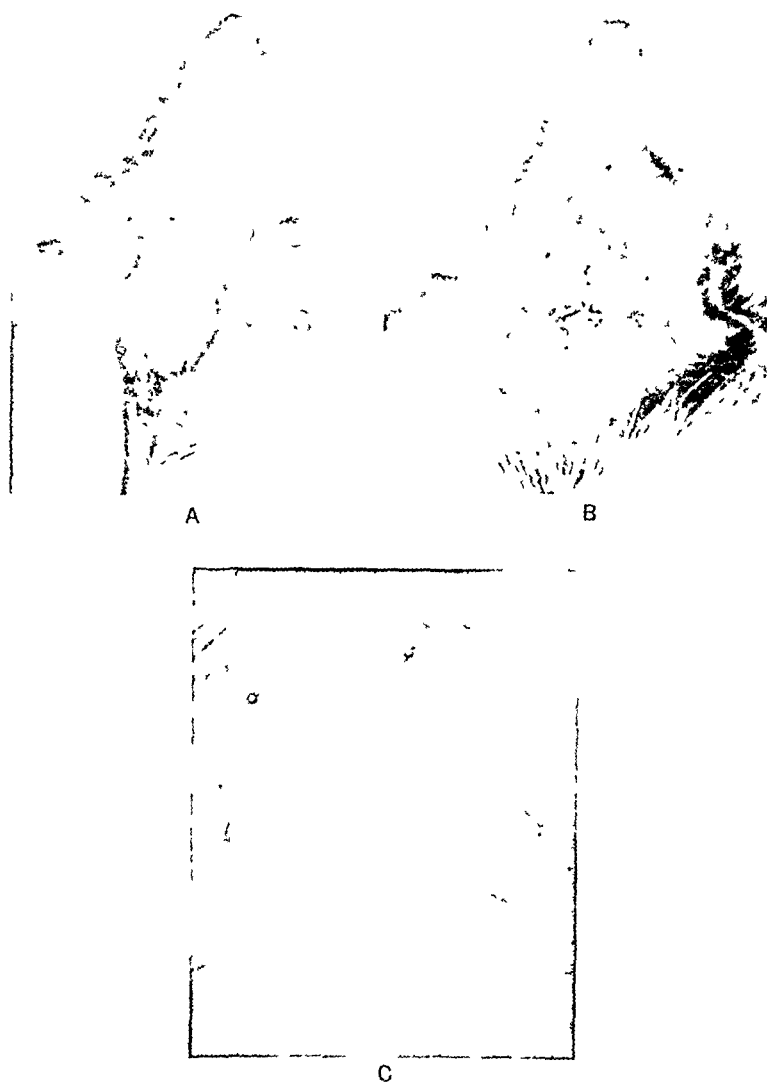
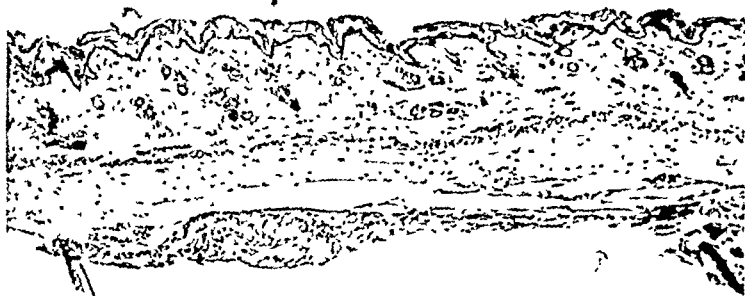


FIG 5 (Experiment 15) a Vitamin B Complex Deficient Rat Thirty days after the application of mustard gas there was a thick crust and circumjacent erythema. The crust was adherent. The scalp was fixed to the skull. b When the adherent crust and circumjacent skin were excised injury to the skull was disclosed. c Normal Rat Thirty days after the application of mustard gas there was contraction of the scar. It was a slightly atrophic pliable superficial scar.

of the cutaneous alteration was very superficial. The skin was not bound to the deeper structures at any time. The skin was loose and movable in the injured area. It was evident that the injury was confined to the skin (Fig. 5c). By contrast there was a different reaction pattern in the deficient rats. In 24 hours after the application there was diffuse erythema of 16 to 20 or more millimeters. The margins were ill defined and difficult to measure. There was diffuse edema in the erythematous area. One or two days later punctate hemorrhage was observed in the central portion. Irregularly shaped crusts appeared 3 or 4 days after the applications. These increased in thickness and in a week or 10 days the crusted areas were adherent to the underlying tissues. The scalp was bound to the skull and was immobile. In the meantime, the peripheral erythema had subsided. When the crust was forcibly removed, it was evident that the injury had extended down to and had involved the skull (Fig. 5a. and b.).

*Microscopic Examination:* The epidermis, cutis, and the sebaceous glands were atrophied in the rats that were deficient in the entire vitamin B complex other than thiamine. The skins of the deficient rats were approximately half as thick as the skin of their normal litter mate controls that received 10 per cent yeast as the source of vitamin B. Biopsy sections were taken on the third, fifth, tenth, eighteenth and twenty-fifth days after the application of mustard gas to the heads. The microscopic findings were consistent with the macroscopic signs of injury. The patterns were strikingly different in the deficient rats and the controls. Signs of injury, as well as signs of healing, were delayed in the deficient rats. Three days after the application of mustard gas to the deficient rats it was difficult to detect any signs of injury other than slight edema in the cutis; the atrophic epidermis, hair follicles and sebaceous glands were undisturbed (Fig. 6a). Sections from the normal rats taken 3 days after the application of mustard gas showed extensive alterations in the epidermis, follicles and cutis. There were intraepidermal vesiculation, destruction of epidermal cells and crusting at the site of application. In the cutis there were edema and diffuse cellular infiltration which was often most extensive in the vicinity of the hair follicles and the sebaceous glands. The blood vessels were dilated and hyperemic (Fig. 6b). Five days after the application of mustard gas to the heads of the deficient rats, there was massive edema throughout the cutis, particularly the lower half (Fig. 7a). The blood vessels were dilated and hyperemic. There were no detectable changes in the epidermis. In the control rats at the same stage there was an increase in the destruction of the epidermis and an increase in the crusting (Fig. 7b). Diffuse cellular infiltration persisted in the cutis. The blood vessels were dilated and hyperemic. In 10 days after mustard gas was applied to the heads of the deficient rats the edema subsided considerably and there were disintegrative alterations of the epidermal cells. There was mild diffuse cellular infiltration in the cutis, and the blood vessels were hyperemic (Fig. 8a). Sections taken from the foreheads of control rats on the tenth day showed inter- and intraepidermal vesiculation and acanthosis (Fig. 8b) in some areas and ulcera-



A



B

FIG. 6 (Experiment 15.) a Showing skin of the forehead of vitamin B complex deficient rat 3 days after application of liquid mustard gas. There is atrophy of epidermis, cutis and sebaceous glands. At this stage very slight edema in the cutis is the only sign of cutaneous injury or response to the application of mustard gas. b. Showing the skin of the forehead of the normal control rat 3 days after application of liquid mustard gas. At this stage there is extensive microscopic evidence of cutaneous injury and response to the application of liquid mustard gas. There are intraepidermal vesiculation and crusting at the site of application. In the cutis there are edema and a diffuse cellular infiltration. The vessels are dilated and hyperemic.



A



B

FIG. 7. (Experiment 15.) a Showing skin of the forehead of vitamin B complex deficient rat 5 days after the application of liquid mustard gas. In the lower half of the cutis there is extensive edema. The blood vessels in the cutis are dilated and hyperemic. The atrophic epidermis at this stage is unaltered. b. Showing skin of the forehead of normal rat 5 days after application of liquid mustard gas. Note the destruction of the epidermis and the thick crust. In the cutis there is a diffuse cellular infiltration which is most extensive in the region of the follicles. The blood vessels are dilated and hyperemic.



A



B

FIG. 8. (Experiment 15.) a. Skin of forehead of rat deficient in the vitamin B complex 10 days after application of liquid mustard gas. The edema has subsided considerably. There are now changes in the epidermis. Hyperemia persists. There is a mild diffuse infiltrate in the cutis. b Skin of forehead of normal rat 10 days after the application of liquid mustard gas. Note the vesicles in the epidermis. There is less edema in the cutis at this stage than there was 5 days previously (Fig. 7b). There has been a decrease in cellular infiltration.

tion and crusting in other areas. The cellular infiltration and edema in the cutis apparently subsided somewhat during the period from the fifth to the tenth day. Eighteen days after the applications there were extensive crusting and transverse tears in the epidermis of the deficient rats. The atrophic hair follicles immediately below the crusted area were unaffected. The edema in the cutis had partially subsided. In the sections taken on the eighteenth day from control rats there was newly formed connective tissue in the upper cutis. In the region adjacent to the injured area there were no signs of alteration. Twenty-five days after the applications there was complete destruction of the epidermis in the deficient rats (Fig. 9a). There were also extensive changes in the connective tissue. There was diffuse cellular infiltration extending into the subcutaneous region. The muscles also were involved. By contrast, in sections (Fig. 9b) taken on the same day from the normal control animals, the microscopic signs of alteration were confined to a localized portion of the skin and healing had occurred. There was restitution of the epidermis. Scar tissue in the upper half of the cutis replaced the hair follicles and sebaceous glands. The lower half of the cutis showed no changes. The muscle was uninvolved. In the regions circumjacent to the injury there were no signs of alteration.

*Experiment 16:* Experiment 15 was repeated on the mid back of 7 deficient rats and 4 controls in order to determine whether the results would be identical in a different location. The results on the back were similar but not quite as striking as those on the head.

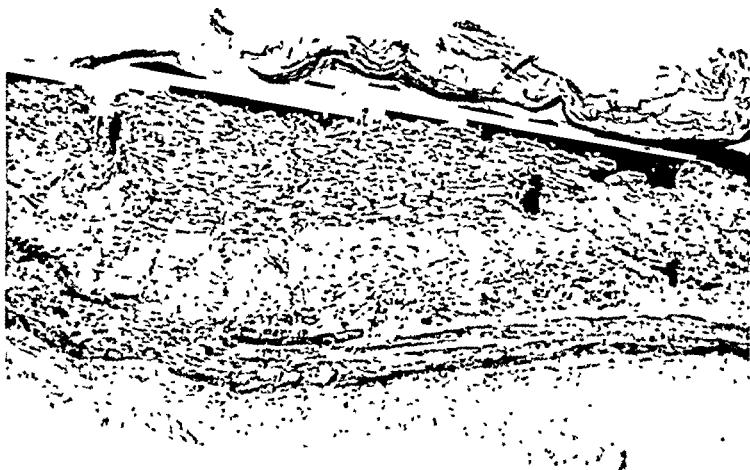
*Conclusion:* The reaction pattern to mustard gas in rats deficient in the entire B complex other than thiamine differed from the reaction pattern in normal rats. In the deficient rats with atrophic skin the injury extended through the cutis, subcutaneous tissue and the muscle. In normal rats the injury was limited to the skin.

#### THE INFLUENCE OF CHOLINE AND CYSTINE

*Experiment 17:* Three litters of young rats were divided into 2 groups, A and B. To the control group A consisting of 6 rats the following diet was fed: casein 20%, sugar 54%, lard 10%, and salts 6%. Five hundred milligrams of cystine was added to each kilogram of the diet. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. Into each kilogram of the ration the vitamin B complex was incorporated as crystalline supplements in the following amounts: thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, pantothenic acid 15 milligrams, nicotinic acid 30 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams. To group B, consisting of 10 rats, was fed a diet and supplements similar to those of group A, except that the supplements of choline and cystine were omitted. Two weeks later



A



B

FIG. 9. (Experiment 15.) a. Showing forehead of vitamin B complex deficient rat 25 days after the application of liquid mustard gas. The atrophic epidermis has been destroyed completely. There are changes in the connective tissue and there is a diffuse cellular infiltration extending deeply into the subcutaneous tissue. The muscle also is involved. b. Showing forehead of normal rat 25 days after the application of mustard gas. In the injured area there is restitution of epidermis. In the upper portion of the cutis there is scar tissue. Note the absence of hair follicles and sebaceous glands in this region. The injury is confined to the skin.

1.77 milligrams of mustard gas was applied to the shaved back of each rat. Comparative weekly measurements for 8 weeks showed that the initial injuries were slightly larger in the rats of group B but healing time was not appreciably affected. Leukocyte counts before and 3 days after the application of mustard gas showed no characteristic differences for the 2 groups.

*Experiment 18:* Two litters of young rats were divided into 3 groups, C, D, and E. To group C, consisting of 6 rats, was fed the diet described in Experiment 17 with the exception that no cystine was added. To group D, consisting of 8 rats, the above diet and supplements were fed and in addition 1500 milligrams of cystine were incorporated into each kilogram of the ration. To group E, consisting of 4 rats, the above diet and supplements were fed and in addition 3000 milligrams of cystine were incorporated into each kilogram of the ration. One month later 1.77 milligrams of mustard gas was applied to the shaved back of each of the rats. Leukocyte counts before and 3 days after the applications showed no characteristic differences in the 3 groups. Comparative weekly measurements for 8 weeks showed no differences in the extent of injury or the healing time in the three groups.

*Conclusion:* The findings in Experiments 17 and 18 indicate that omission of choline and cystine from synthetic diet results in slight enhancement of mustard gas injuries; the omission of cystine from the diet had no influence on the extent of injury. Healing time is not affected by omission of choline and cystine together or cystine only. Large doses of cystine added to a synthetic diet failed to accelerate healing.

#### MAGNESIUM DEFICIENCY

*Experiment 19:* Magnesium deficiency was produced in 4 litters of young rats by feeding the following diet: casein 200 grams, dextrin 541 grams, butter fat 100 grams, McCollum salts (No. 62) 52 grams. Crystalline supplements of 20 milligrams of thiamine, 20 milligrams of riboflavin, 10 milligrams of pyridoxine, 30 milligrams of nicotinic acid, 15 milligrams of calcium pantothenate, 1 gram of choline hydrochloride, 375 milligrams of inositol, and 375 milligrams of para-amino benzoic acid were incorporated into the diet. Five hundred milligrams of cystine, 18,200 i.u. of vitamin A and 5,460 i.u. of vitamin D were included in the ration. The control diet differed from the deficient diet in that 1 gram of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  was triturated into the salt mixture. The cutaneous signs of magnesium deficiency consist of generalized severe erythema in some animals and multifomed erythematous plaques in others in the early stage, and eczematous dermatitis, edema and thickening of the skin in late stages (9). Liquid mustard gas was applied to erythematous and non erythematous area, to edematous areas and to areas of fissured eczematous dermatitis. There was considerable variation



in the results which were not reproducible. In some animals erythema and/or edema seemed to enhance injury due to mustard gas, but in other animals injuries in erythematous and/or edematous skin were less extensive than those in apparently normal skin areas of magnesium deficient animals as well as in the normal controls. When mustard gas was applied to areas of eczematous dermatitis the injury was not enhanced.

*Conclusion:* The findings in this experiment were inconclusive. (See discussion.)

#### THE EFFECT OF FEEDING PARA-AMINO BENZAMIDE

*Experiment 20:* To one group of adult rats the following diet was fed: casein 20%, sugar 54%, lard 10%, and salts 6%. Five hundred milligrams of cystine were added to each kilogram of the diet. Oleum percomorphum and alpha tocopherol supplied vitamins A, D, and E. Into each kilogram of the diet the vitamin B complex was incorporated as crystalline supplements in the following amounts: thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, pantothenic acid 15 milligrams, nicotinic acid 30 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams. To a similar group of rats was fed the same diet with the exception of para-amino benzamide. Weight curves were approximately equal in the 2 groups. One and seventy-seven hundredths milligrams of mustard gas was applied to the shaved back of each rat. The extent of injury and healing time were equal in each group. The leukocyte counts before and after the burns showed no characteristic differences in the 2 groups.

#### THE LACK OF INFLUENCE OF MASSIVE DOSES OF CRYSTALLINE VITAMIN B SUPPLEMENTS ON THE EXTENT AND HEALING TIME OF CUTANEOUS INJURY

*Experiment 21:* To one litter of 7 carefully matched young rats the following diet was fed: casein 20%, lard 10%, sugar 54%, and salts 6%. The ration was supplemented with oleum percomorphum and alpha tocopherol to supply vitamins A, D, and E. Five hundred milligrams of cystine was added to each kilogram of ration. The litter was divided into two groups: A, consisting of 3 rats and B consisting of 4 rats. Into each kilogram of the diet of the control group (A) the vitamin B complex was incorporated as crystalline supplements in the following amounts: thiamine 20 milligrams, riboflavin 10 milligrams, pyridoxine 10 milligrams, pantothenic acid 15 milligrams, nicotinic acid 30 milligrams, choline 1000 milligrams, inositol 375 milligrams, para-amino benzoic acid 375 milligrams. Into the diet of the other group (B) supplements of thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, inositol, and para-amino benzoic acid were incor-

porated at a level five times as great as that in group A. Two weeks later 1.18 milligrams of mustard gas was applied to the back of each rat. All the rats survived. They were observed for 2 months and comparative measurements were made weekly. The extent of injury and the healing time were identical in each group.

*Conclusion:* A five fold increase of an adequate supplement of thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid inositol, and para-amino benzoic acid failed to accelerate healing of mustard gas burns.

### DISCUSSION

Analysis of the results of the above experiments indicates that atrophy and inanition were factors which enhanced injury and resulted in delayed healing. With the exception of the inconclusive results in the biotin deficient rats, the severity of injury and delay in healing were proportional to the degree of atrophy of the skin. The most severe burns were sustained by the rats that had been deprived of the entire vitamin B complex other than a small supplement of thiamine which was necessary for the maintenance of life (Experiment 15). In this experimental deficiency there is marked atrophy of skin and its appendages and subcutaneous fat is practically absent. Inanition is most pronounced in this combined deficiency. The manner in which liquid mustard gas penetrated through the atrophic skins was in sharp contrast to its action in the skin of the normal control rats where much of the agent apparently was fixed, and the resulting inflammatory reaction was limited to the skin and the subcutaneous tissue. The penetration was of such depth in the scalps of the deficient rats that not only was the musculature involved but there was also necrosis of the skull. Such penetration could be explained simply on a structural basis, that is on the thickness of the skin, but it seems more likely that the atrophic, deficient skin was unable to "fix" the agent because of profound chemical changes secondary to the deficiency. The protective function of epidermal cells and skin appendages is tremendous when the skin maintains its integrity. Subcutaneous fat has been found to possess many biochemical properties and the lipids of fat deposits are constantly subject to a variety of chemical reactions of synthesis, degradation and interconversion

(10). The absence of subcutaneous fat conceivably could account for some of the lack of protection other than by its structural presence. Next in severity to vitamin B complex deficient group were the burns of the riboflavin deficient rats (Experiment 8). The skins of riboflavin deficient rats also are extremely atrophic and subcutaneous fat is sparse. Quantitatively, however, the atrophy and decrease of subcutaneous fat in riboflavin deficient rats is not as severe as in the rats deficient in the entire vitamin B complex (other than thiamine). The other members of the vitamin B complex, namely, pyridoxine, pantothenic acid, nicotinic acid, choline, inositol and para-amino benzoic acid are included in the riboflavin deficient ration and probably are utilized to an extent sufficient to impart to the skin a measure of protective and healing qualities not possessed by the rats deficient in the entire vitamin B complex other than thiamine. Inanition is a prominent symptom of riboflavin deficiency but the general condition and survival of riboflavin deficient rats are better than in the combined deficiency. Next in severity were the burns inflicted on rats with severe protein deficiency (Experiment 5). The skins were atrophic, but were not as thin as the skins of the rats deficient in the entire vitamin B complex other than thiamine and the riboflavin deficient rats. Inanition is not as marked either. Fat deficiency (Experiment 6) should be listed next in the order of deficiencies causing enhancement of injury and delay in healing. The skin in fat deficiency is atrophic but the estimated atrophy is less marked than in the three preceding deficiencies, and the general condition is much better as they survive for many months. Pyridoxine deficiency (Experiment 9) was next in order. In pyridoxine deficiency the skin is only slightly atrophic in the areas tested, that is, on the back, and although underweight their general condition is fairly good until the late stage of the disease. Last in the list of conditions showing a clear cut effect on the extent of injury and healing time was pantothenic acid deficiency, in which atrophy of the skin and loss of subcutaneous fat is also slight, and incidentally in this deficiency there is less evidence of inanition than in the other vitamin B complex deficiencies. Carbohydrate deficiency, vitamin A deficiency, magnesium deficiency and protein deficiency of mild degree exerted no influence on the burns. In these conditions there is only slight atrophy except in the late stages of the deficiencies and inanition is not prominent. In biotin deficiency there is atrophy

of the skin in the fully developed stage of the disease which follows variable stages of hypertrophy of the skin (11). The findings in the biotin deficiency experiment (Experiment 13) are difficult to explain because during the advanced stage of the deficiency, in which a thick coating of scales, crusts, fat and broken hair shafts covered the back (11), some protection was afforded against small doses of liquid mustard. What was responsible for the protection was not determined. When the coating was scraped from the biotin deficient rats and smeared on normal rats it failed to afford protection. In adult rats with mild biotin deficiency the injuries were slightly larger than those in normal controls and healing was slightly delayed. The significance of the findings in biotin deficiency cannot be explained on the basis of the relation of the degree of atrophy to the severity of the injury, as the atrophy in the advanced stage of the deficiency in the young rats was more marked than the degree of atrophy in the mild stage of the disease in adult rats. The findings in the magnesium deficient rats (Experiment 19) were inconclusive, but demonstrated that liquid mustard gas burns were not consistently influenced in either direction by edema, vascular dilation or vesicular dermatitis. These manifestations of inflammation were expected to cause aggravation of injury, but there was great variation in the reactions produced in different groups and the patterns were not reproducible. The lack of aggravation in areas of eczematous dermatitis is difficult to explain as it would be reasonable to predict larger and more slowly healing burns in previously damaged skin. Signs of transient pantothenic acid deficiency were manifest in carbohydrate deficiency when the deficiency of carbohydrate was not compensated for by protein and fat. Signs of pantothenic deficiency have been reported in connection with zinc poisoning (12). The toxicity of mustard gas may have increased the pantothenic acid requirement. However, the results of Experiment 10 indicate that pantothenic acid is less important than riboflavin and pyridoxine in contributing to the protection against and the healing of the vesicant injury.

#### SUMMARY

1. The application of small measured amounts of liquid mustard gas to the skin of rats results in uniform, reproducible injuries which may be measured precisely and utilized for experimental studies.

2. The pattern of injury in human skin which is manifested by vesiculation differs from that in rat skin in which gross vesiculation never occurs. Hypersensitivity to subsequent exposure of human skin to mustard gas frequently results; hypersensitivity in rats cannot be produced unless previously injured skin is deliberately reinjured.

3. The extent of cutaneous injury is increased and healing is delayed in rats conditioned by deficiencies of the entire vitamin B complex (other than thiamine), riboflavin, pyridoxine, pantothenic acid, protein (severe) and fat. Carbohydrate, vitamin A and mild protein deficiencies exert no influence on the extent of injury or healing time. Cystine, choline and para-amino benzamide had no effect on the healing of mustard gas injuries. Five fold increases of adequate crystalline vitamin B supplements failed to accelerate healing.

4. Inanition and atrophy of the skin appear to influence the extent of injury and the healing time of liquid mustard gas injuries. The severity of injury and delay in healing were proportional to the degree of atrophy of the skin and the grade of inanition. The results in biotin deficient animals were inconclusive and not consistent with the apparent relationship of injury and healing to atrophy and inanition.

#### BIBLIOGRAPHY

1. DAVIS, M. I. J.: The Dermatologic Aspects of the Vesicant War Gases. *J. A. M. A.* 126: 209, Sept. 23, 1944.
2. SULLIVAN, M., AND NICHOLLS, JR.: The Nutritional Approach to Experimental Dermatology. Introduction and Review of Literature. *J. Invest. Dermat.* 3: 309, Aug. 1940.
3. KENTON, H. B.: The Arthus Phenomenon in the Rat. *J. Infect. Dis.* 69: 238, 1941.
4. SULLIVAN, M.: Unpublished data.
5. a) LONGCOPE, W. T.: Insusceptibility to Sensitization and Anaphylactic Shock. *J. Exper. Med.* 36: 627, 1922.  
b) OPIE, E. L.: Inflammatory Reaction of the Immune Animal to Antigen (Arthus Phenomenon). *J. Immunol.* 9: 231, 1924.  
c) CANNON, P. R., AND MARSHALL, C. E.: Studies on the Mechanism of the Arthus Phenomenon. *J. Immunol.* 40: 127, 1941.  
d) COCA, A. F., RUSSELL, E. F., AND BAUGHMAN, W. H.: The Reaction of the Rat to Diphtheria Toxine. *J. Immunol.* 6: 387, 1921.
6. SULLIVAN, M., AND NICHOLLS, J.: Nutritional Dermatoses in the Rat. IV. Riboflavin Deficiency. *J. Invest. Dermat.* 4: 181, June 1941.

7. SULLIVAN, M., AND NICHOLLS, JR.: Nutritional Dermatoses in the Rat. I. Vitamin B<sub>6</sub> Deficiency. *J. Invest. Dermat.* 3: 317, Aug. 1940.
8. SULLIVAN, M., AND NICHOLLS, JR.: Nutritional Dermatoses in the Rat. VI. The Effect of Pantothenic Acid Deficiency. *Arch. Dermat. & Syph.* 45: 917, May, 1942.
9. SULLIVAN, M., AND EVANS, V. J.: Nutritional Dermatoses in the Rat. X. A Comparison of Experimental Magnesium Deficiency and Disseminated Neurodermatitis. *Arch. Dermat. and Syph.* 49: 33, Jan., 1944.
10. FAWCETT, D. W.: Differences in Physiological Activity of Brown and White Fat as Revealed by Histochemical Reactions. *Science.* 105: 123, Jan. 31, 1947.
11. SULLIVAN, M., AND NICHOLLS, J.: Nutritional Dermatoses in the Rat. V. Signs and Symptoms Resulting from a Diet Containing Unheated Dried Eggwhite as the Source of Protein. *Arch. Dermat. & Syph.* 45: 295, Feb. 1942.
12. GROSS, P., HARVALECK, Z., AND RUNNE: Vitamin Deficiency Syndrome in the Albino Rat Precipitated by Chronic Zinc Chloride Poisoning. *J. Invest. Dermat.* 4: 385, Oct. 1941.

# COMPARATIVE EFFICIENCY OF SINGLE AND MULTIPLE DOSAGE REGIMENS OF THE PENICILLINS<sup>1</sup>

CHARLES G. ZUBROD<sup>2</sup>

*Department of Pharmacology and Experimental Therapeutics, and Department of  
Medicine, The Johns Hopkins University*

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Current practice in the use of penicillin G for the treatment of infections in man demands that a relatively constant blood concentration be maintained. This is generally accomplished by the administration of frequent doses of penicillin G in aqueous solution or by one dose per day of penicillin in oil and beeswax. Both methods are painful for many of the patients, and present other drawbacks such as waste of nursing time, expense, etc. It is pertinent therefore to consider the experimental basis for such modes of administration, in order to appraise how essential is the need for a constant penicillin blood level for the treatment of bacterial infections.

There are three reports available concerning the effectiveness of infrequent dosage of aqueous penicillin upon bacterial infections. Tillet, Cambier and McCormack (1) showed that pneumococcal pneumonia in man could be cured by penicillin, even though the drug was omitted for a 12-hour period during the night. Jawetz (2), using a crude penicillin of unstated composition in the treatment of a hemolytic streptococcus infection in mice, showed that its antibacterial effect lasted eight hours, while penicillin was measurable for only one hour. He suggested a trial of less frequent administration of penicillin in human infections. White, Lee and Alverson (3) found that a single dose of penicillin X would cure 100 per cent of mice with C 203 hemolytic streptococcus peritonitis. White (4), in similar studies with I. M. penicillin X, observed no superiority of multiple dose schedule over single doses.

These reports indicate that in vivo the penicillins exert an antibacterial effect for a longer period than can be explained on the basis of

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<sup>2</sup> Roche Fellow in Medicine and Experimental Therapeutics.

the persistence of the penicillin blood concentration. No reports were found which would support the hypothesis that aqueous penicillin G was more efficient as an antibacterial agent either in animals or man, when given at frequent intervals, than when the same total amount of penicillin was given at infrequent intervals. Experiments were therefore undertaken to investigate the comparative efficiency of various dosage regimens of penicillin, using as the test object a virulent hemolytic streptococcus infection in mice, and the pure penicillins G and K as the curative agents. It is to be emphasized that in these experiments, the total amount of penicillin administered to the mice is kept constant, and that only the division of the total dose is varied.

#### METHODS AND MATERIALS

1. *Mice.* The mice used for the infections were the CFW strain from Carworth Farms, of uniform stock and age. The weight range for all the mice used was 13–23 grams. However, the weight ranges for the individual experiments were narrower, the figures for the five experiments being 15–20 g., 14–19 g., 14–18 g., 15–23 g. and 13–16 g.

2. *Hemolytic Streptococcus Infection.* The mice were infected with strain C 203 of a group A,  $\beta$ -hemolytic streptococcus. Stock cultures were grown in trypticase-soy-phosphate rabbit-blood broth, and were kept at 6°C. Virulence was maintained by mouse passage at 2–3 day intervals. Weekly titration of the virulence in mice showed that one organism (or chain), injected intraperitoneally, routinely killed in 24–48 hours. In the experiments mice were infected intraperitoneally with 1.0 ml. of a  $10^{-5}$  dilution of a 5 hour blood broth subculture. Such a dilution contained 1200 to 4500 organisms and killed 78 of 80 control mice in 16 hours  $\pm$  5 hours. The 2 remaining control mice died on the 5th and 7th days respectively. Culture of the heart blood in each instance grew out hemolytic streptococcus. Since it required between 1 and 2 hours to infect 400 mice, in each experiment the first 10 mice and the last 10 mice infected were held as untreated controls.

In the 5th experiment, shown in Table 4, the mice received 12,000 organisms, instead of the average of 2300 for the other 4 experiments.

300–400 Mice were infected for each experiment. In any single experiment 20 mice were used as a unit for each alteration in dosage schedule or amount of dosage. Groups of 4–7 such treatment units



were run for each dosage schedule; and 3-4 dosage schedules were tried in each experiment. In a representative experiment, for example, 6 groups of 20 mice would receive a single dose of penicillin G in amounts ranging from 0.06-2.0mg./k.; 6 groups would receive 3 doses at 8 hour intervals, 6 groups would receive 8 doses at 3 hours intervals, and so on. Reproducibility of results was shown to be of a high order by treatment in each experiment of groups of mice on either a single dose or 3 doses at 8 hour intervals.

TABLE 1  
*Data on source and purity of penicillin salts used in the experiment*

PENICILLIN SALT	LOT NUMBER	SOURCE	IN VITRO POTENCY* UNITS PER MG.	DIFFERENTIAL RATIO <i>B. subtilis</i> <i>S. aureus</i>	COUNTER-CURRENT INTERPRETATION
Sodium G	J-85-4320	Lilly thru Anti- biotic Com- mittee U.S. P.H.S.	—	—	3% K types 2.3% X type Trace F type
Sodium G	V-31	Squibb	1690	0.98	93.5% peni- cillin G
Sodium K	7/12/46	Pfizer thru Antibiotic Committee U.S.P.H.S.	2075	0.36	95% penicil- lin K
Ammonium K	Do-320-K	Squibb	2100	0.36	5% other K types

\* *S. aureus* cup test.

Only those mice still alive 21 days after completion of treatment were counted as survivors. Cultures were made from heart blood of about half the mice dying later than day 7, and in each instance hemolytic streptococcus grew out.

3. *Penicillins*. Crystalline salts of penicillin G and penicillin K, in aqueous solution, were used for the treatment of the infected mice. *No penicillin in oil and beeswax was employed.* The purity of the samples was greater than 93 per cent for each salt as shown by the Craig method of counter-current distribution. The data for the 4 salts are shown in Table 1. All the mice in table 3 were treated with penicillin

G-Lilly J-85-4320. Another penicillin G, Squibb V-31, with extensive documentation of purity, showed in vivo no statistically significant difference from the Lilly sample (Table 2).

The penicillin salts were kept in a desiccator at 6°C. Fresh samples were weighed out, on the day preceding the experiment, and were dissolved in 0.9 per cent sterile saline a few hours before use. Injections were made intramuscularly in 0.2 ml. volumes. Treatment was started, in all but one experiment, 4 hours after infection. In the experiment in Table 4, treatment was begun immediately after infection. Dosage was computed in terms of milligrams of penicillin salt per kilogram of mouse.

TABLE 2

*Comparative in vivo Activity of Two Samples of Pure Penicillin G*

Organism: Hemolytic streptococcus, Group A, Strain C 203.

Infection: Intraperitoneal, 1.0 ml. of  $10^{-5}$  dilution of 5 hour culture, containing 1200 organisms.

Mice: CFW, 15-23 g.

Treatment: Intramuscular sodium penicillin G Lilly J-85-4320 and Squibb V-31 in 0.2 ml. volume. Started 4 hours after infection. Schedule 3 equal doses at 8 hour intervals.

TOTAL AMOUNT OF SODIUM PENICILLIN G ADMINISTERED	PROPORTION OF SURVIVORS		
	Untreated	Treated with Penicillin G Lilly	Treated with Penicillin G Squibb
mg./k.			
1.0	0/20	19/20	17/19
0.5		19/20	16/20
0.25		5/20	5/20
0.12		1/20	1/20

## RESULTS

1. *Multiple Dose Regimens of Penicillin G.* The effect of the various dosage regimens of penicillin G upon the survival rate of infected mice is shown in Table 3. It is important to point out that reading across the table for any mg./k. dosage the total amount of penicillin administered is the same, and only the division of total amount is varied. The comparative efficiency of the different dosage schedules is arbitrarily made on the basis of the calculated Median Survival Dose (SD50). The method of Litchfield and Fertig (5) was employed for the calculation of the SD50 and of the standard error.

The results of the single dose of penicillin G are of such a character

that they cannot be compared with the results of multiple dose schedules, and will be considered separately. The SD50s for the multiple dose regimens, as shown in Table 3, do not differ significantly with the exception of Schedule 5. Hence it seems valid to conclude that, with regard to multiple dose regimens of penicillin G, survival of the mice depends upon the total dose administered. Within certain, but

TABLE 3

*Comparative in vivo Efficiency of Various Dosage Regimens of Sodium Penicillin G*

Organism: Hemolytic streptococcus, Group A, Strain C 203.

Mice: CFW, 13-23 grams.

Infection: Intraperitoneal, 1.0 ml. of  $10^{-5}$  dilution of 5 hour culture, containing 1200 to 4500 organisms.

Treatment: Intramuscular sodium penicillin G, Lilly J-85-4320, in 0.2 ml. volume, 4 hrs. after infection.

TOTAL AMOUNT OF SODIUM PENICILLIN ADMINISTERED	PROPORTION OF SURVIVORS ON SCHEDULES NUMBER						
	Un- treated	I Single Dose	II 4 Doses at 24 Hr. Intervals	III* 3 Doses at 8 Hr. Intervals	IV† 3 Doses at 8 Hr. Intervals	V 8 Doses at 3 Hr. Intervals	VI 8 Doses at 1 Hr. Intervals
mg./k.							
8	0/80	14/20					
4		36/40	18/20				
2		32/39	17/19	17/20	17/20	19/20	
1		27/40	18/20	18/20	38/40	20/20	19/20
0.5		26/40	18/20	18/20	38/40	15/20	20/20
0.25		20/40	5/20‡	8/20	11/40‡	0/19‡	8/20
0.12		8/20		0/20‡	3/40‡	2/20‡	1/20
0.06				0/20‡		2/20‡	1/20
SD 50 (mg./k.)		0.25	0.32	0.29	0.27	0.45	0.24
Standard Error $\pm$ mg./k.		0.27	0.03	0.03	0.03	0.04	0.03

\*  $\frac{1}{2}$  of total dose given at start, remainder divided into  $\frac{1}{4}$ - $\frac{1}{4}$ .

† 3 equal doses at 8 hr. intervals.

‡ Deaths of > 10 per cent of mice before completion of treatment.

fairly broad limits, the survival rate is not affected by the number of fractions into which this total dose is split, nor by the interval between doses. When the total dose, as shown by Schedule 5, is split into 8 fractions and given at the rate of  $\frac{1}{8}$  of the total dose every 3 hours, this limit has been passed, and significantly larger amounts of penicillin G are required to save half the mice.

There is one variable which prevents precise comparison of the dosage regimens in the lower dose ranges (0.25 mg./k. or less). With Schedules 2, 4 and 5, as shown by the asterisks in Table 3, significant numbers of mice began to die before the total dosage could be completed. However, the 21-day survival rates for these schedules are

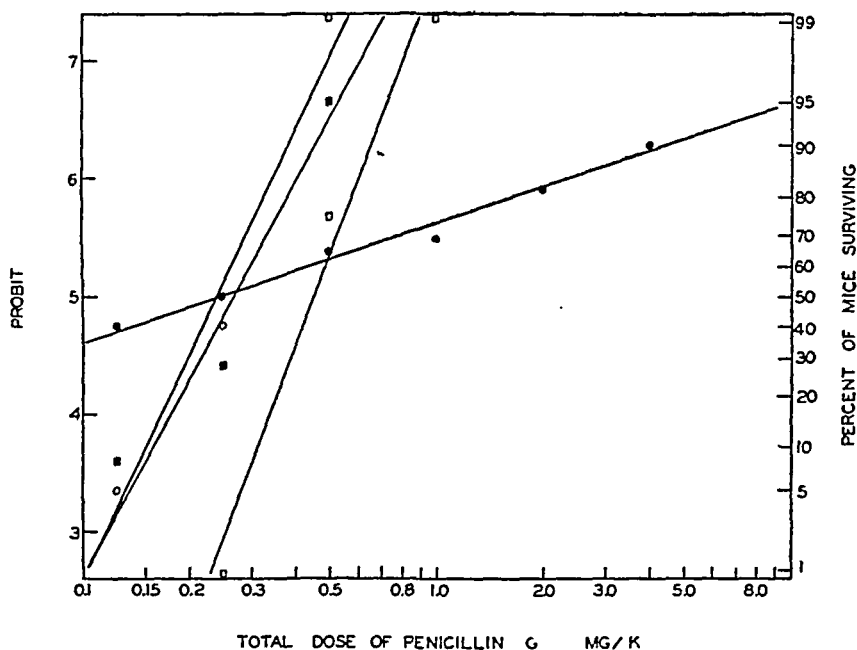


FIG. 1. Effect of intramuscular penicillin G upon survival rate of mice infected with hemolytic streptococcus. Data taken from Table 3.

- single doses
- 8 equal doses given at one hour intervals
- 3 equal doses given at 8 hour intervals
- 8 equal doses given at 3 hour intervals

not substantially different from those of schedules 3 and 6, where the mice completed treatment.

*2. Single Dose of Penicillin G.* The survival rate of mice, treated with various single and multiple dose regimens of penicillin G, is shown in Table 3 and in graphic form in Figure 1. It is noted that a single dose gives a flat type of dosage response curve, i.e., for each increment

TABLE 4

*Effect of immediate treatment with sodium penicillin G upon therapeutic effectiveness*

Organism: Hemolytic Streptococcus, Group A, Strain C 203.

Mice: CFW, 13-16 g.

Infection: Intraperitoneal, 1.0 ml. of  $10^{-6}$  dilution of 5 hour culture, containing 12000 organisms.

Treatment: Intramuscular, sodium penicillin G Lilly J-85-4320, in 0.2 ml. volume.  
Treatment started immediately after infection.

TOTAL AMOUNT OF SODIUM PENICILLIN G ADMINISTERED	PROPORTION OF SURVIVORS		
	Untreated	Single Dose	3 Doses Given At 8 Hr. Intervals
mg./k.			
4	0/20	16/20	
2		16/20	20/20
1		12/20	20/20
0.5		7/20	19/20
0.25		11/20	3/20
0.12		4/20	0/20
0.06		0/20	0/20

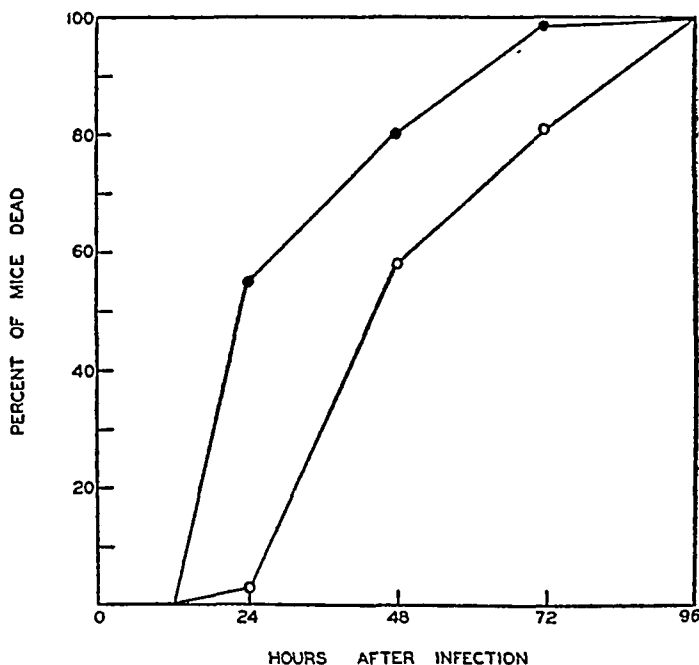


FIG. 2. Survival times of mice with hemolytic streptococcus infection, receiving 0.25 mg./k. of penicillin G in a single dose ○—○ and in all various multiple dose schedules of Table 3, ●—●. Only mice dying within 96 hours after infection have been charted.

in dosage there is only a small gain in the number of mice surviving. If the SD50 is calculated for this curve, it is the same as the SD50 for multiple doses, but the error for the single dose SD50 is so large that it is not valid to compare the different types of curves. The same type of flat curve results whether the animals are treated immediately or after a 4 hour incubation (Table 4).

TABLE 5

*Comparative in vivo Efficiency of Various Dosage Regimens of Penicillin K*

Organism: Hemolytic streptococcus, Group A, Strain C 203.

Mice: CFW, 13-23 g.

Infection: Intraperitoneal, 1.0 ml. of  $10^{-6}$  dilution of 5 hr. culture, containing 1200-4500 organisms.

Treatment: Intramuscular sodium penicillin K, Pfizer 7/12/46, and ammonium penicillin K, Squibb DO-320-K, in 0.2 ml. volumes 4 hrs. after infection. Run as part of experiments together with penicillin G, and hence controls are not repeated in this table.

TOTAL AMOUNT OF PENICILLIN K ADMINISTERED	PROPORTION OF SURVIVORS			
	I Single* Dose	II 4 Doses at* 24 Hr. Intervals	III 3 Doses at† 8 Hr. Intervals 1/2-1/4-1/4	IV† 8 Doses at 1 Hr. Intervals
mg./k.				
16	19/20		19/20	
8	20/20	18/20	14/20	16/20
4	19/20	12/20	8/20	7/20
2	11/20	1/20	2/20	0/20
1		0/20	1/20	1/20
SD50 (mg./k.)	1.9	4.0	5.0	5.1
Standard Error $\pm$ mg./k.	0.04	0.06	0.12	0.04
SD50 K	7.6	12.5	17.2	21.0
SD50 G				

\* Sodium penicillin K, Pfizer, 7/12/46.

† Ammonium penicillin K, Squibb Do-320-K.

Several observations may be made about the effect of a single dose of penicillin G upon hemolytic streptococcus infection of mice.

A. Even with a dose of 8 mg./k., a significant number of mice fail to survive.

B. On the lower end of the curve, the converse is true. That is if 0.12 mg./k. is given as a single dose, 40 per cent of the mice survive; but when the same quantity is divided into 3 to 8 doses, only an occasional mouse survives.

C. In the first 24 hours after a single dose of 0.25 mg./k. of penicillin G, there are practically no deaths (figure 2). With various multiple dosage regimens at 0.25 mg./k., 55 per cent of the mice are dead at the end of 24 hours.

3. *The Dosage of Penicillin K.* Penicillin K was given to several groups of mice, and the results are shown in Table 5. These mice were treated as part of the same experiments in which penicillin G was used, so that the experimental conditions were identical. The 2 different samples of penicillin K appeared to have the same activity and the results have been pooled.

The SD50 for the multiple dosage regimens is 13 to 21 times that for penicillin G. There is only a slight variation in the SD50 ratio of K to G, with the various modifications in dosage schedules. Not enough data have been collected to decide whether the dosage response curve for a single dose of penicillin K is also flat. The standard error for the SD50 of a single dose of penicillin G is so large that the SD50 ratio of K to G is meaningless.

#### DISCUSSION

In these experiments with a fulminating streptococcus infection in mice the controlling factor in survival rate is the total dosage of the penicillins. Penicillin G and penicillin K have approximately the same effectiveness as antibacterial agents in mice when given every 8 or 24 hours as when given every hour. Penicillin G blood levels in the mouse, after single intramuscular doses of 4.5 mg./k. and 3.0 mg./k. are maintained for 30 minutes to 1 hour (2, 6). (Valid measurement of penicillin G in the presence of serum cannot be made below a concentration of 0.1 unit per ml. (7)). Comparable doses in man maintain a level above 0.1 unit per ml. for 4 hours (8). On the basis of the data in mice, it seems clear that the antistreptococcal effect of aqueous penicillin G outlasts the measurable blood levels by many hours. In mice, therefore, nothing is gained by administering penicillin G more often than once every 24 hours.

With a given amount of penicillin G, what is the most efficient schedule of administration in hemolytic streptococcal infections in mice? No single answer can be given. A multiple dose schedule would have to be adopted, as a single dose could not be expected to give 100 per cent cures with any reasonable dose. However, a large initial

dose confers an advantage not given by schedules in which the total dose is fractioned into many equal parts. This advantage consists in the fact that a large initial dose protects mice with this fulminating infection for the next 24 hours, whereas with multiple dose schedules, many mice die before they receive enough drug to bring the infection under control.

No final explanation can be assigned for the flat dosage response curve obtained with a single dose of penicillin G. Perhaps it is due to the possibility, as pointed out by Bigger (9) that in any bacterial population a certain percentage of the organisms are in a resting phase. If penicillin G is unable to affect such phases it may be that the flat curve is merely a measure of the number of organisms in the resting phase.

Penicillin K in these experiments has an activity about  $\frac{1}{17}$  that of penicillin G. This ratio varies little with alteration in dosage schedule.

These results with penicillin G in mice cannot be directly transferred to man. Nevertheless, it is clear that there is a persistent antibacterial effect of penicillin G, long after its disappearance from the blood. Hence there is little logic in the use of constant blood concentration as the basis for the dosage regimen of penicillin G. Since no one wants to know the minimal effective dose of penicillin in severe infections in man, one should proceed in trial of the above principles in human infections with the use of excess penicillin. Some early results of Tillett et al. (1), in the treatment of pneumococcal pneumonia, indicate that infrequent dosage of penicillin is safe, and probably just as effective as the eight-injections-a-day routine. On the basis of fewer injections for the patient and less work for the nursing staff, one might try, in the treatment of penicillin-susceptible infections, 0.18 g. (300,000 units) of penicillin G intramuscularly every 12 hours. In patients where treatment is begun late and the bacterial population presumably high, it would be important to try the effect of "large" initial doses, say 0.6 gram (1,000,000 units) I. M., immediately and again in 8 or 12 hours, and then smaller doses at 12 hour intervals.

#### SUMMARY

1. In the treatment of hemolytic streptococcus infections of mice with multiple doses of aqueous penicillin G or of penicillin K, survival



of the mice depends on the total dose administered over a fairly broad range of dosage schedules.

2. Single doses of penicillin G give a flat type of dosage response curve with a large standard error. These results cannot be validly compared with those of multiple dose regimens.

3. Large initial doses of penicillin G are advantageous in so far as they protect mice for a much longer period than multiple small doses.

4. The *in vivo* activity of pure penicillin K in streptococcal infection in mice is  $\frac{1}{18}$  to  $\frac{1}{20}$  that of pure penicillin G.

5. Suggestions are made for the applications of these experimental data to the treatment of bacterial infection in man.

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#### REFERENCES

1. TILLET, W. S., CAMBIER, M. J., AND McCORMACK, J. E.: *Bulletin N. Y. Acad. Med.*, **20**: 142, 1944.
2. JAWETZ, E.: *Arch. Int. Med.*, **77**: 1, 1946.
3. WHITE, H. J., LEE, M. E., AND ALVERSON, C.: *Proc. Soc. Exp. Biol. & Med.*, **62**: 35, 1946.
4. WHITE, H. J., Personal communication, 1946.
5. LITCHFIELD, J. T., JR. AND FERTIG, J. W.: *Bulletin Johns Hopkins Hospital*, **69**: 276, 1941.
6. RICHARDSON, A. P., WALKER, H. A., MILLER, I. AND HANSEN, R.: *Proc. Soc. Exp. Biol. & Med.*, **60**: 272, 1945.
7. TOMPSETT, R., SHULTZ, S., AND McDERMOTT, W.: *J. Bact.*, **53**: 581, 1947.
8. McDERMOTT, W., BUNN, P. A., BENOIT, M., DuBOIS, R., REYNOLDS, M. E.: *J. Clin. Invest.*, **25**: 190, 1946.
9. BIGGER, J. W.: *Lancet*, **2**: 497, 1944.

## THERAPEUTIC CONFERENCE<sup>1</sup>

THE JOHNS HOPKINS SCHOOL OF MEDICINE AND THE JOHNS HOPKINS  
HOSPITAL

Conferences on therapy have been held on alternate Saturdays during 1946-47 and are a joint endeavor of the Departments of Medicine and of Pharmacology and Experimental Therapeutics. The approach to the problems of treatment is based on presenting the fundamental mechanisms of the disease concerned, the physiological action of the pharmacological agents which are used in the treatment of that disease, and our clinical experience in the management of the patient. This rather vertical approach to problems of clinical therapeutics is an effort to place our management of the treatment of the patient on as scientifically sound and rational a basis as possible. Stenotyped reports have been made of these conferences, and a few have been selected for publication in the Bulletin.

### THE TREATMENT OF HEART FAILURE

#### PART I: DIGITALIS AND ITS DERIVATIVES

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*Dr. A. McGehee Harvey:* The conference this morning will be the first of two on various aspects of treatment of heart failure. Today we will take up digitalis and its derivatives and their use in clinical medicine. Dr. Andrus is going to talk briefly first of all about the physiology of the myocardial defect causing congestive heart failure.

*Dr. E. Cowles Andrus:* Preliminary to discussion of digitalis therapy I propose to rehearse some of the physiological factors which are the basis of what we call congestive heart failure. It is due, at least in its beginning, to the fact that the heart muscle is incompetent to perform effectively the mechanical work imposed upon it. It is a state of diminished mechanical efficiency. The efficiency of any pump or engine is determined, as you recall, by its supply of energy and by its loading. The supply of energy for the myocardium comes to it by

<sup>1</sup> Presented on March 15, 1947 at The Hurd Memorial Hall, The Johns Hopkins Hospital.

way of the coronary arteries; the energy transformations are measurable in terms of oxygen consumption. The energy supply is dependent in some measure upon the integrity of the heart as a pump since this organ provides its own blood flow. The loading is determined by the inflow through the great veins and by the peripheral resistance; the efficiency relative to the loading is influenced by two particular factors, the nervous control of the heart which mediates certain unloading reflexes, and the inherent property of the heart muscle which makes it exquisitely adaptable within certain limits to the load that is thrust upon it.

The response of the heart, efficient and inefficient, to imposed load may be displayed in an isolated denervated preparation, the heart-lung preparation. It is not suggested that the physiology of myocardial insufficiency is altogether as simple as this, but the inherent properties of the heart muscle are the same in the heart-lung preparation and in situ. Moreover there is clinical evidence that "the Law of the Heart" does apply under normal, and under some abnormal, conditions.

In the classic diagram with which you are familiar, the efficiency and the oxygen consumption of the heart-lung preparation are plotted against its work output. With increasing load, oxygen consumption rises; it is almost a linear function of the diastolic length of fiber, or ventricular volume. With increasing load too, the length and the tension of fiber at the beginning of contraction increases. Up to an optimum limit this results in an increased efficiency: increase in work output per unit of energy. However, when the optimum is exceeded, the curve of efficiency flattens out or falls so that beyond the optimum, when additional work is imposed upon the myocardium it is accomplished only at the expense of an extravagant oxygen consumption. This is the essential functional fault in myocardial insufficiency.

Although it is fundamental, this is not the only element responsible for the clinical picture of congestive heart failure. As the methods of clinical physiology have been applied to this condition they have not exonerated the heart muscle but have demonstrated additional abnormalities, such as abnormal salt excretion by the kidney, increased blood volume, etc., which combine to produce the picture and which sometimes dominate it. It is proposed to lift the myocardial fault

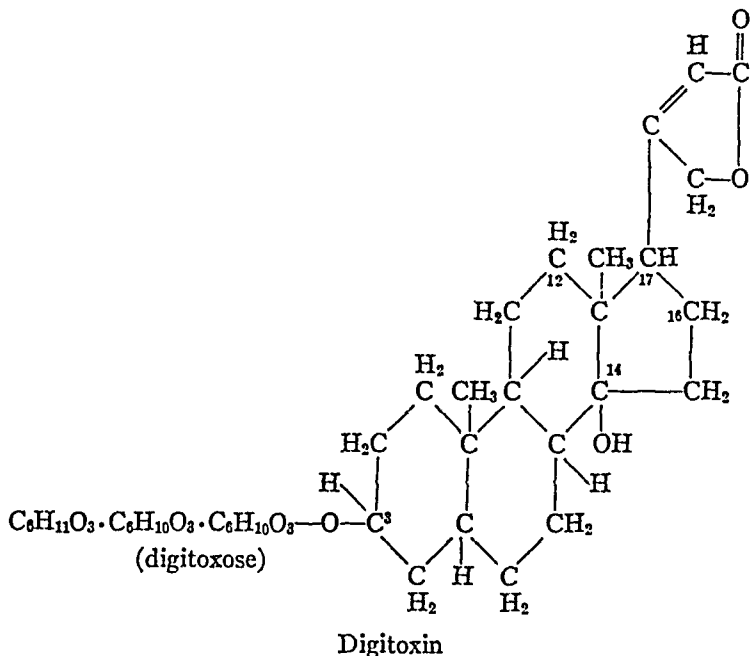
out of its context this morning and to discuss it and its treatment separately.

*Dr. Harvey:* Dr. Butler is now going to discuss the comparative effectiveness of the various digitalis-like preparations.

*Dr. Thomas C. Butler:* A digitalis-like action is produced by a number of naturally occurring products, comprising glycosides, alkaloids, and toad venoms. Of these only the glycosides have found any practical use in clinical medicine. Cardioactive glycosides occur in numerous plants. About 30 glycosides have now been isolated in crystalline form and have received some pharmacological study. Only a few of these have been used even experimentally in man. In the last few years several glycosides have become available in pure form in sufficient quantities to allow thorough clinical study. This discussion will be concerned more with the specific differences between some of these glycosides than with the traditional pharmacological effects of digitalis.

The cardioactive glycosides are all closely related chemically, being hydroxylactones of sterol hydrocarbons in which one hydroxyl group is connected with a sugar molecule or a chain of several sugar molecules. The non-carbohydrate part of the molecule is called an aglycone or genin. In 1934 the relation of the cardiac glycosides to the sterols and bile acids was discovered. In the following year or two the advances in the knowledge of the structures were very rapid since much of the knowledge that had been laboriously accumulated for years could be immediately fitted in with the new ideas of the basic structures. The formula of digitoxin is shown as a representative of the type. From the clinical point of view this is one of the most important of the available pure glycosides, although it is now known that digitoxin itself is not one of the native glycosides of *Digitalis purpurea*. A glycoside containing one more molecule of glucose occurs in the leaf and is readily converted to digitoxin by an enzyme also present in the leaf. The aglycones of the other glycosides are quite similar in structure. Several different sugars are found in the glycosides. The glycosides show considerable structural specificity. The genins on intravenous injection have qualitatively the same type of action as the glycosides, but the sugar nevertheless has an important effect. The genins are less active than the glycosides, are not active when given

by mouth, and do not show the delay in onset of action or the difficultly reversible fixation to heart muscle characteristic of the glycosides. The unsaturated lactone ring in the genin is essential for activity. Saturation of the double bond or opening the ring by hydrolysis destroys activity. The hydroxyl in position 14 is also apparently essential for activity. The spatial configuration of the steroid ring system is likewise important. Such apparently minor changes of structure as the introduction of an hydroxyl group in digitoxin in position 16 to



form gitoxin or in position 12 to form digoxin, although not qualitatively impairing cardiac activity, do alter the quantitative behavior in the body in an important manner that will be discussed later.

The high degree of structural specificity in a rather complex molecule has defeated attempts to synthesize cardiotonic substances. Simple lactones patterned after that part of the molecule lack the characteristic action of digitalis. Progress has been made on the partial synthesis of aglycones from intermediates derived from bile acids and cholesterol, but the synthetic substances so far produced

lack the specific structural features that appear to be essential for activity. Some synthetic derivatives of natural genins have been made in which a carbohydrate other than the naturally occurring one has been attached to the hydroxyl at position 3 or in which that hydroxyl has been esterified with simple carboxylic acids. These compounds are active and are of theoretical if not of practical interest.

The quantitative study of the absorption, excretion, and distribution of the glycosides is handicapped by the lack of chemical methods of sufficient sensitivity. The total dose of digitoxin required to digitalize a man is of the order of 1 mg. A very delicate method indeed would be required to detect such a substance in the concentrations in which it would be present in blood and tissues. Yet without chemical determination and solely by the observation of the physiological effects in appropriate experiments, a surprising amount of information has been gathered about the behavior of these substances in the body.

The various cardiac glycosides, although having qualitatively similar actions on the heart, differ greatly in the following properties, which are of great importance in determining the method of clinical use:

- (1) inherent potency
- (2) absorption from the intestine
- (3) rapidity of onset of action
- (4) persistence of action.

The completeness of absorption from the intestine can be judged by comparing the oral with the intravenous dose producing the same effect. It has been found that digitoxin produces almost identical effects by the two routes both in laboratory animals and in man. It must therefore be nearly all absorbed. Ouabain, on the other hand, is almost totally ineffective by mouth. This probably indicates that little is absorbed, although the relative ineffectiveness or oral doses might be in part attributable to the short duration of action of any material that is absorbed. Most of the other glycosides that have been studied in this way appear to be only partially absorbed. The table shows these relationships.

It can be shown on the isolated frog heart or on the mammalian heart-lung preparation that glycosides added to the perfusing liquid do not act immediately. This latent period is different for different glycosides, being much longer for digitoxin than for ouabain. This

time may be the time necessary for the fixation of the drug to the tissue or the delay in the biological response after fixation. If the glycosides are injected intravenously in man, similar differences in the time of onset of action can be observed, the extremes again being represented by digitoxin and ouabain. If ouabain is injected intravenously, changes in the electrocardiogram, in venous pressure, and in the ventricular rate in auricular fibrillation can be detected within a very few minutes and reach their maximum within about half an hour. On the other hand, the effects of intravenous digitoxin come on very much more slowly. They may not be detectable in less than an hour and may reach their maximum only after 12 hours or longer.

TABLE 1\*

*Showing Quantitative Differences in the Pharmacological Behavior of some of the Pure Glycosides as Observed in Man*

GLYCOSIDE	INTRAVE- NOUS DIGI- TALIZING DOSE	ORAL DIGITALIZING DOSE	DAILY MAIN- TENANCE DOSE	TIME BEFORE MAXIMUM EFFECT OF INTRAVE- NOUS DOSE	DURATION OF ACTION DAYS
	mgm.	mgm.	mgm.	hrs.	
Digitoxin.....	1.2-2.0	1.2-2.0	0.1 -0.4	2-24	14-21
Digoxin.....	1.0-2.0	4-8	0.25-0.75	$\frac{1}{2}$ -2	5-7
Digilanid C.....	1.0-2.0	5-20	0.5 -2.0	$\frac{1}{2}$ -2	3-21
Ouabain.....	0.5-1.0	not effective	0.25-0.5	$\frac{1}{2}$ -2	2-3

\* This table is based on that of Vander Veer, Med. Clin. North Am., 30, 1263 (1946), but contains modifications and additions from a number of other sources.

In isolated heart preparations it can be shown that the effects of the glycosides do not disappear rapidly when glycoside-free perfusing liquid is introduced. This phenomenon is usually attributed to the difficultly reversible fixation of the drug to the tissue, but could conceivably be due to the persistence of the physiological effect after the disappearance of the drug. Again, digitoxin and ouabain are the extreme examples, the former washing out the most slowly and the latter the most rapidly. In man the glycosides show the same relation. The effects of a single dose of digitoxin may persist for as long as 3 weeks, while the effects of a dose of ouabain last only a few hours. This persistence of action is the basis of the usual dosage schemes for initiating and maintaining the desired effect.

Thus, the relatively minor differences in chemical structure found in the various cardiac glycosides, although not modifying essentially the qualitative action on the heart, are associated with important quantitative differences in absorption, onset of action, and persistence of action; and it is these differences that determine the field of clinical usefulness of the drugs. For instance, a drug like digitoxin with its complete absorption and persistent action is suited for the routine maintenance of digitalization by oral dosage, while a drug like ouabain is only useful for the rapid initiation of an effect by intravenous injection.

One of the jobs that has always been considered the rightful province of the pharmacologist is the biological assay of digitalis preparations. Almost every common laboratory animal has been used for the assay. Much attention has been given to the standardization of the minutest details of the technical procedures, and the relative merits of the various methods have been argued bitterly. But little attention has been given to the fact that the bioassay of digitalis is a logical impossibility. This is a fact that cannot be evaded by any amount of technical refinement or statistical analysis. Digitalis is a mixture of active principles, and the assay of any such mixture involves either of two assumptions: (1) that the proportions of the several active principles are the same in all samples of the drug; or (2) that the ratio of the therapeutic activity in man to the activity on the test object is the same for each of the active principles. Neither of these assumptions is justified for digitalis. Obviously the amounts of digitoxin and ouabain that are equivalent on intravenous injection in a cat in the official U.S.P. assay cannot be therapeutically equivalent on oral administration to a man.

I do not intend to make the unqualified statement that the bioassay of digitalis has served no useful purpose. Perhaps it has, and perhaps for practical purposes it is good enough. It probably has reduced to some extent the variation of the products on the market. In the usual method of treatment, digitalis is in effect assayed on each individual patient. Large variations would be troublesome, but a very precise knowledge of potency is unnecessary. Particularly in digitalis from the same commercial source, the variations are probably of little practical importance.



Some at least of the logical objections to the assay of digitalis on laboratory animals could be met by performing the assay on man. This is a method that has been advocated by Gold, electrocardiographic changes being used as the criteria of drug action. Whether this procedure would be feasible for large scale use is problematical.

The only logically satisfactory solution to the problem of bioassay of a mixture of active principles is to use one of the pure substances in medicine and so dispense with the necessity of assay altogether. It is to be hoped that this will eventually be the solution of the digitalis problem. Several of the pure glycosides are now commercially available. It is not in my province to pass on the desirability of substituting one or more of these for the crude leaf in medical practice. Some cardiologists think that pure glycosides, particularly digitoxin, do have advantages over the crude mixture. To the pharmacologist it would be gratifying not to have to sacrifice his intellectual integrity by doing a logically impossible bioassay, but this is of course not a sufficient reason. The principal drawback at present to the use of a pure glycoside is economic. The patient buying his drugs on prescription will pay about three times as much for digitoxin as for an equivalent amount of digitalis leaf; but even so his daily maintenance dose of digitoxin will usually only cost him 3 to 10 cents, not a very large item in the budget of many a private patient. The difference assumes more important proportions, however, in the annual budget of a large charity hospital which can buy the crude leaf in large quantities at a very low price. In these institutions, until the price of pure glycosides is considerably reduced, much more convincing arguments would have to be presented for the abandonment of crude digitalis than any theoretical cavilling on the part of a pharmacologist.

*Dr. Harvey:* Dr. Andrus will now discuss the clinical use of digitalis.

*Dr. Andrus:* I left the subject of myocardial insufficiency with the heart-lung preparation, and I want to pick it up again there, showing some of the effects of the exhibition of digitalis to such a preparation. In a relatively acute experiment in which myocardial efficiency has fallen to a low level the exhibition of ouabain, for example, increases the work output, the oxygen consumption and the calculated efficiency of the heart. In another preparation in which the diastolic volume of the heart, and therewith its oxygen consumption, has been main-

tained constant, efficiency and work are also increased by ouabain. It has been demonstrated by Starr, among others, that if the volume of the heart is estimated from its frontal area, and the cardiac output is measured, and the work calculated, under normal circumstances there is a direct, apparently optimal, relation between the volume of the heart and the work of the left ventricle. It is also demonstrable that in cases with cardiac disease—some compensated, others uncompensated—the output of work relative to the cardiac volume is diminished.

The clinical effects of digitalis are known to you all. It has a most striking influence upon the rate of ventricular beat in the presence of auricular fibrillation. This effect is certainly not solely the result of vagus stimulation. This chart is taken from a report by Cushny in 1925. It shows the fall of ventricular rate in a patient with auricular fibrillation in two experiments separated by a suitable interval. Strophanthin was given on both occasions, and in the experiment represented by the dotted line it was rapidly followed by an intravenous dose of 2 mg. of atropine. Slowing took place just the same whether the vagus influence was present or not. Another, again one of Cushny's, shows that as the full effect of digitalis is achieved the increase in rate following atropine diminishes. This is restored again after the digitalis effect is permitted to wear off. It can be demonstrated that the acceleration of ventricular rate by exercise is similarly suppressed by full digitalization. This is a point of considerable practical importance in estimating and maintaining the full effect of digitalis. It is best to be certain that the patient with auricular fibrillation receives sufficient digitalis to maintain a ventricular rate within reasonable limits at tolerable activity rather than simply at rest.

I mentioned the fact that cardiac volume and output were optimally related. A second, and probably principally important, effect of digitalis upon the human heart is to increase its systolic contraction and frequently to diminish its size. If 1.8 gm. of digitalis is administered to a patient with fully compensated rheumatic heart disease without evident cardiac enlargement the frontal area of the cardiac shadow in the teleoroentgenograms may be shown to diminish. The effect upon the work and the function of this heart may not be entirely advantageous. The venous pressure may alter very little but circulation

time may rise and cardiac output fall considerably. These are not changes which one would ordinarily associate with improvement of the circulation. In cases of rheumatic heart disease with myocardial insufficiency, there may again be a reduction of the cardiac area and volume after digitalization. But here venous pressure often falls and cardiac output rises. Body weight decreases as diuresis occurs. These changes, it can be shown, bring the relation of cardiac size to cardiac work back toward or into the zone of normal.

In considering the action of digitalis upon the circulation, it is sometimes difficult to distinguish cause and consequence. For example McMichael and Sharpey-Schafer have compared the effects of venesection with those of the intravenous injection of digitoxin. Both procedures bring about a reduction in right auricular pressure and an increase in cardiac work. The same effects can be obtained by trapping blood in the extremities behind tourniquets. The increase in cardiac work with venesection is less than that produced by the digitalis glycosides and more transient. The arterial blood pressure falls following venesection; when digitalis is administered the blood pressure is maintained. These authors interpret their data as showing that digitalis produces its benefit by some effect upon venous inflow and right auricular pressure, reducing the loading of the machine to within tolerable limits. Alternatively the effect might be explained, it seems to me, by an improvement in the competence of the ventricles to put out the blood which is brought to them.

Now nobody knows, as far as I am aware, precisely how these effects of digitalis are brought about. In that sense its administration is still covered with some degree of empiricism, but certain verities have, I think, developed in the course of years. The indication for the use of digitalis, you will all agree, I am sure, is congestive heart failure. Some of the empiricism which characterizes the use of digitalis conceivably results from the fact that patients are not frequently examined or are examined with insufficient care. Digitalis should not be given on a symptomatic basis alone, but only with a full comprehension of the clinical status of the patient. As to choice of preparation, Dr. Butler's remarks are important and enlightening. I personally have had more experience with the powdered leaf than with any other preparation, but digitoxin more recently introduced has certain impressive advantages.

The occasion for the administration of either digitoxin or ouabain by vein is, in my opinion, rare. The emergencies in which lives are saved by this technique are relatively infrequent. Also it is infrequently advantageous to administer a full digitalizing quantity of the drug in a single dose by mouth. In rare instances in which vomiting is frequent, it is advisable to start administering digitalis in the form of cedilanid intramuscularly or digitoxin intravenously in divided doses. Whenever possible it is preferable to rely on the oral route and usually to digitalize in 24 to 48 hours, observing the effect with care.

I think that we would all give digitalis more cautiously if we had to take it ourselves. I remember some very unhappy days which followed my taking 1.4 gram of digitalis in 24 hours. This is an experience that has ever since influenced the rapidity with which I have administered digitalis to a patient. It is not a completely innocuous drug.

As a standard to go by I suggest that it is poor technique to digitalize a patient and to obtain toxic symptoms if it can possibly be avoided. The symptoms of digitalis intoxication are familiar to all of you. The earliest is anorexia. Nausea and vomiting follow soon thereafter and are persistent and annoying. With the typical bigeminal irregularity of the cardiac rhythm, you are again familiar, and, not as a toxic symptom but as a manifestation of the direct action of digitalis, the change in the electrocardiogram.

I prefer to leave the remainder of the session for questions if you care to ask them. One final point I would like to mention which may be brought up again in another connection: the unappreciated danger of the production of overdigitalization if diuresis is produced too rapidly in a fully digitalized patient.

*Dr. Harcey:* The meeting is now open for questions. I would like to ask Dr. Andrus to give us a resumé of just how he approaches digitalizing a patient—how much to give and with what rapidity to give it.

*Dr. Andrus:* Considering the use of digitalis as one of several items of treatment let's take a patient with hypertension who has received no digitalis and has acute pulmonary edema. Here I think that one would give a full dose of strophanthin intravenously. It may be that as my experience develops—the experience of others may be greater with it—a relatively large dose of digitoxin would be indicated and again intravenously. Digitoxin does have the important advantage

in contrast to strophanthin that you can translate its effect directly in terms of oral digitoxin if you want to follow with the latter.

Now as to the patient who is orthopneic, breathless and edematous, brought into the hospital having received no digitalis. If the patient is not vomiting I would try to digitalize by mouth in 24 to 48 hours in divided doses. Probably again digitoxin will replace the other products. Personally I would not rush to intravenous digitoxin in that individual if he could take it by mouth. It may be possible that the effects could be more easily achieved that way. I just have an aversion to intravenous medication.

*Question:* As to figuring out the total dose that is going to be necessary, of course some have complicated rules but we have translated those into more practical rules of thumb. Does that still hold with these new preparations? What is the relative dosage?

*Dr. Andrus:* I think others who have had more experience with digitoxin may be able to answer that better than I but in the case of the powdered leaf I aim with the ordinary sized individual at 1.2 gram. With digitoxin I suppose it would be 1.2 milligram. However, there is a considerable degree of variation among patients as to the amount required to achieve full therapeutic effect. The physician in charge must guide therapy with digitalis by watching the patient and examining him, stopping short if toxic symptoms supervene or pushing if the effects are not achieved. Finally one has to admit that, sometimes, without the other armamentarium for treating cardiac failure which will be mentioned in more detail in the next session, digitalis is relatively ineffective. You have certainly had that experience. In these cases one can be misguided into producing intoxication because of the lack of therapeutic result. Specifically there is an average but one shouldn't be completely limited by trying to be too quantitative about it.

*Dr. Harvey:* Does the absorption of digitoxin take place in the presence of passive congestion in the gastro-intestinal tract the same as in the normal individual?

*Dr. Andrus:* As far as I know. Certainly in some of these patients with auricular fibrillation and congestive failure who have been studied very carefully ventricular rate does go down and to just about the same extent with intravenous and oral doses.

*Dr. Harvey:* Dr. Baker, have you any comments to make?

*Dr. Benjamin M. Baker, Jr.:* It is refreshing to hear of a tendency to digress from the practice of precise calculation of digitalis dosage. Patients are individuals and the administration of digitalis to them should be individualized. When digitalis is indicated the largest amount of drug that can be tolerated without signs or symptoms of intoxication should be our goal. One can calculate roughly a safe fraction of the optimal dose and give that rapidly. Having warned the patient of the earliest symptoms of intoxication the remainder can be given in small, frequently repeated amounts.

I would like to encourage the more frequent use of digitalis in patients with enlarged hearts which have not yet failed as one means of postponing the onset of decompensation.

*Dr. Harvey:* Of course, there were some clinicians who believed that digitalis was not indicated in the absence of auricular fibrillation; it was withheld from patients with heart failure with normal sinus rhythm. I think that idea has gradually disappeared. In certain cases with sinus rhythm it is extremely useful and should be given. The tendency has indeed gone further, and in cases with a severe degree of hypertension even though there is no clinical manifestation of failure except reduction in cardiac reserve, one tends to give an estimated digitalis dose. That is the subject upon which there is a great deal of disagreement. Perhaps Dr. Andrus has something to add.

*Dr. Andrus:* I am very grateful to you for starting to answer this question. It is one of the most contentious points in the whole subject. I think that there is a trend in treating patients who have cardiac disease but not obvious congestive failure, to explore the possible advantages of digitalizing gradually and maintaining digitalization. This procedure must be fitted to the evident needs of the individual patient. I don't think that one can grant the same amount of justification to the use of digitalis in surgical cases prior to operation simply because the pulse rate is rapid. I am not convinced of the value to anyone of the use of digitalis under those circumstances. On the other hand I believe that if one accepts, as one must, the fact that digitalis can, in the experiment in the heart-lung preparation, reverse and maintain the reversal of a rapidly developing insufficiency, it must be assumed that the drug can maintain cardiac efficiency *in vivo*.

Particularly in cases of hypertension with cardiac enlargement I think there is increasing justification as you say, Dr. Harvey, for examining the benefit of digitalis.

*Dr. Charles R. Austrian:* I have nothing particular to say except to emphasize what has been said already by Dr. Andrus that, in my experience at least, the frequency of the need for rapid digitalization by vein is generally over-emphasized, for to the majority of patients one can give sufficiently rapid and adequate dose of the drug by mouth. I agree with you that mathematical precision in dosage has been replaced with profit by a "rule of thumb" in many cases. Though for rapid digitalization a total dose of 0.1 gm. per 10 lbs. of body weight suffices when from 25 to 50% of this amount is given initially, I would like to reemphasize the need of individualization in the therapy, to insist that to assume that one can calculate accurately in advance the optimum dosage is misleading, and to point out that maintenance of digitalization is scarcely less important than is the establishment of digitalization.

I think a point raised by Dr. Baker has been bypassed, namely, the so-called tonic medication with digitalis in the sense in which that term was utilized by Dr. Henry Christian. In the prophylactic therapy of the hypertensive heart, it was his idea that there may be sufficient encroachment on the myocardial reserve without the usual objective evidences of myocardial weakness or of failure to constitute an indication for the administration of the drug in doses of 0.05 to 0.1 gm. once or twice daily—an amount insufficient generally to cause unpleasant symptoms. Now whether or not with such minimal dosage, with an amount of the drug insufficient to cause objectively demonstrable digitalis effect in these patients, one accomplishes his purpose, is a moot point and clinical opinion is divided sharply. Different men hold to different ideas. Starting with a skeptical view of the method of Dr. Christian of what might be termed subdigitalizing a hypertensive heart without signs of failure, I have come to believe it has a distinctly beneficial effect.

*Dr. Baker:* There is important evidence which supports the position Dr. Austrian has taken. Starr has provided convincing proof of digitalis value, in subdigitalizing amounts, by observing serial improvement in the form of ballistocardiograms of patients being slowly digitalized.

Equally significant are the observations of Harrison that attacks of paroxysmal dyspnoea are less frequent in patients receiving digitalis, even in subdigitalizing amounts, than in patients not receiving the drug at all.

I would like to take exception to the position Dr. Andrus took regarding the management of cardiac patients who must be operated upon. No matter how trivial the circulatory abnormality or the operation may be, one can never predict what emergency may arise. Considerable amounts of electrolyte solutions or whole blood may be urgently required postoperatively. It has been amply demonstrated that parenteral fluids may raise the venous pressure of individuals with normal circulations. Therefore to my mind, patients with potential congestive heart failure are in a much better position to face the surgeon and the possibility of urgent intravenous therapy if they are preoperatively digitalized, or at least partially so.


*Dr. Harvey:* This is not now so urgent as it was before these newer preparations were available. Now we are able to digitalize if necessary very rapidly.

It is one of the most puzzling problems of digitalis therapy that the maintenance dose may vary so tremendously. You see those who take as much as 3 tenths gram of powdered leaf a day and those who get toxic symptoms on 1 tenth of a gram daily. One of the greatest difficulties arises in attempting to determine that level in each patient without having an elusive effect of digitalis on the patients who do require more than the estimated dose of a tenth of a gram per day.

*Dr. Andrus:* I didn't mean to deny digitalis to a patient going through surgery. I meant particularly that it shouldn't be given for tachycardia alone. All that Dr. Baker has said of course is completely justified. It underscores one other thing: that the use of digitalis requires thorough evaluation of the clinical status of each patient. Wherever possible the venous pressure, circulation time, and the vital capacity should be measured.

*Dr. Harvey:* Dr. Andrus, I would like to ask a question regarding what I think is another of the difficult situations in which the average student and house officer finds himself. What is the place of digitalis in the treatment of the cardiac arrhythmias, aside from its effect upon the congestive heart failure which may result from such arrhythmias when prolonged?



*Dr. Andrus:* In the presence of auricular fibrillation you will probably get some control of the irregular rapid ventricular rate by the use of digitalis. This is even more striking in the presence of failure. In the presence of hyperthyroidism and auricular fibrillation you will get relatively little effect from digitalis. In the presence of auricular flutter the effect of digitalis is to increase the rate of the circus movement to an extent to which the auricle cannot respond coordinately and auricular fibrillation supervenes. In the process of digitalization you gain control over the ventricular rate. Frequently, although not invariably, this causes the circulatory status of the patient to improve.  It is not always sufficiently appreciated that digitalis may contribute to the evolution of cardiac arrhythmia which is itself a handicap. Extrasystoles which are so frequent that they produce a considerable pulse deficit are certainly no advantage to circulation. In ventricular tachycardia digitalis is contraindicated; it is likely to make matters worse rather than better. One of the manifestations of severe digitalis poisoning is ventricular fibrillation. I have seen such cases which I am sure resulted from mistaken use of digitalis. In one the patient took an overdose by error.

*Dr. Harvey:* How about the situation in patients with myocarditis as far as digitalis is concerned?

*Dr. Andrus:* It would be my impression that if you are satisfied with that diagnosis digitalis would not be indicated.

*Dr. Harvey:* Of course that brings up the problem of the place of digitalis in the treatment of severe infections including pneumonia. Is there any increased susceptibility to digitalis in these people?

*Dr. Andrus:* I can't prove it but it is my impression that there is. I think oxygen would do the heart more good than digitalis in those circumstances.

*Dr. Baker:* The use of digitalis in pneumonia remains a controversial subject. Twenty years ago in this hospital it was given to almost every patient with lobar pneumonia. Gradually we have developed a clearer understanding of the common circulatory disturbance in this important disease; peripheral circulatory failure is relatively common and congestive heart failure is rare. Digitalis is contraindicated in peripheral circulatory failure and hence is rarely indicated in patients with lobar pneumonia unless they have pre-existing cardiac disease.

Dr. Andrus has pointed out that digitalis produces certain cardiac arrhythmias. So does anoxemia and the digitalized, cyanotic patient with lobar pneumonia or bronchial asthma is a good candidate for cardiac arrhythmias. Before giving digitalis to patients with either of these conditions I advocate consideration, among other things, of frequent determinations of the venous pressure.

*Dr. Austrian:* Opinion concerning the indications for and the efficacy of digitalis in acute lobar pneumonia has varied greatly through the last few decades, influenced especially when the differentiation was established between the circulatory failure of peripheral as distinguished from that of cardiac origin. Some forty years ago in this clinic a man's tenure as a house officer was jeopardized if he gave digitalis to a patient with lobar pneumonia as a routine procedure, that is in the absence of some identifiable evidence of myocardial embarrassment. Tachycardia alone—without arrhythmia newly developed, falling blood pressure, etc.—was not regarded sufficient indication for exhibition of the drug. That practice prevailed until some short while after work from the Hospital of the Rockefeller Institute seemed to establish the desirability of administering digitalis to every patient with lobar pneumonia, when it was exchanged for such routine administration. Then, once again after it had been established that the drug did not influence the toxic tachycardia and that it even lessened the minute volume output of the undamaged myocardium, routine administration was frowned upon and individualization of the medication was established as the proper method of treatment. From this vacillation there developed the need first to establish the circulatory efficiency; secondly, if there was circulatory failure whether it was of peripheral vascular or of cardiac origin; and thirdly, only if evidence of myocardial defect were developing or apparent was digitalization to be induced.

*Dr. Harvey:* Does anyone else have a question?

*Dr. Nathan B. Herman:* Will you comment on the use of digitalis in bronchial asthma in the absence of true cardiac damage.

*Dr. Andrus:* Speaking in the abstract, I think they ought to have all the support they can get. That is one of the instances in which it might be an advantage rather than a disadvantage to use digitalis.

*Question:* What about the use of digitalis in the failing heart undigitalized but with many ventricular extrasystoles?

*Dr. Andrus:* You not infrequently see that the extrasystoles diminish as digitalis takes effect under those circumstances.

*Dr. Baker:* I would like to put in a plea for the use of quinidine under those circumstances.

*Question:* Is there anything against the use of quinidine with digitalis with heart failure in the presence of extrasystoles?

*Dr. Andrus:* Not that I know of. There is one situation in which we used to feel that the two shouldn't be given together because of their antagonistic action on the auricular muscle; when we were trying to revert auricular fibrillation.

*Dr. Harvey:* I am one of those people who still considers quinidine a dangerous drug.

*Dr. Andrus:* So am I.

*Dr. Baker:* I would like to add further emphasis to the need for caution in the use of quinidine. Although I am sure there is danger in the use of quinidine I am equally afraid of ventricular extrasystoles in a patient who has recently had myocardial infarction. I certainly advocate the administration of quinidine to patients in this fix and the withholding of digitalis as long as possible because of the increased ventricular irritability it may induce.

*Dr. Harvey:* To attempt to revert auricular fibrillation of long standing with quinidine is, I think, bad procedure for several reasons. In almost no instance does the rhythm revert to normal and remain so. The patient may be very little better off as far as the cardiac function is concerned.

**Summary:** In the course of the conference, pharmacologists and clinicians have discussed one of our most useful drugs. The discussion has brought out the lack of knowledge of the mechanism of the action of digitalis despite the fact that it has been extensively employed for more than 150 years in the treatment of "cardiac dropsy". Digitalis has been prepared for use in increasingly pure form, but the dose which produces beneficial effects and that which causes toxic symptoms are still inconveniently close together. Repeated attempts have been

made to design a convenient routine for digitalis therapy. As matters stand at present, to obtain its full benefit the administration of digitalis must be guided by careful preliminary evaluation and periodic re-evaluation of the patient's condition.

The digitalis bodies provide a challenging field for collaborative study by pharmacologists and clinicians. It is not too much to hope that by such future collaboration the mechanism of the action of these important compounds may be elucidated and their usefulness correspondingly increased.

### SELECTED REFERENCES

- (1) CUSHNY, A. R.: *The Action and Uses of Digitalis and Its Allies*. Longmans, Green and Co., 1925.
- (2) EICHNA, L. W. AND TAUBE, H.: Comparison of Actions of Four Cardiac Glycosides on Patient with Congestive Failure. *Am. Heart J.*, **26**, 631, 1943.
- (3) FREEDBERG, A. S. AND ZOLL, P. M.: Digitalis. *New Eng. J. Med.*, **235**, 938, 1946.
- (4) GOLD, H.: Choice of Digitalis Preparations, *Conn. State Med. J.*, **9**, 193, 1945.
- (5) GOLD, H., CATTELL, M., MODELL, W., KWIT, N. T., KRAMER, M. L., AND ZAHN, W.: Clinical Studies on Digitoxin with Further Observation on Its Use in Single Average Full Dose Method of Digitalization. *J. Pharm. and Exp. Therap.*, **82**, 187, 1944.
- (6) LA DUE, J. S., AND FAHR, G.: Effect of Intravenous Administration of Lanatoside C upon Output, Diastolic Volume and Mechanical Efficiency of the Failing Heart. *Am. Heart J.*, **25**, 344, 1943.
- (7) MCMICHAEL, J., AND SHARPEY-SCHAFER, E. P.: Action of Intravenous Digitoxin in Man. *Quart. J. Med.*, **13**, 123, 1944.
- (8) MOE, G. K., AND VISSCHER, M. B.: Studies on Native Glucosides of Digitalis Lanata with Particular Reference to Their Effect upon Cardiac Efficiency and Their Toxicity. *J. Pharm. and Exp. Therap.*, **64**, 65, 1938.
- (9) STEWART, H. J., ET AL.: Action of Digitalis in Compensated Heart Disease. *Arch. Int. Med.*, **62**, 547, 1938.
- (10) STEWART, H. J., ET AL.: Action of Digitalis in Uncompensated Heart Disease. *Arch. Int. Med.*, **62**, 509, 1938.
- (11) STROUD, W. D. AND VANDERVEER, J. B.: Six Year Study of Clinical Efficacy of Various Digitalis Preparations. *J. A. M. A.*, **109**, 1808, 1937.

# THERAPEUTIC CONFERENCE THE TREATMENT OF HEART FAILURE

## PART II. THE USE OF DIURETICS

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*Dr. Harvey:* As a continuation of the last conference on the treatment of congestive heart failure, in which digitalis was discussed, we are now turning to the question of diuretic therapy. Dr. Newman will open with a discussion of the physiology and pharmacology of diuretic drugs.

*Dr. Elliott V. Newman:* The first thing necessary in a discussion of diuretic drugs in cardiac failure is to define what we mean by the word diuresis. As clinicians concerned with diuresis we are primarily interested in removal of fluid from the body, producing negative fluid balance. The abnormal collection of body fluid in cardiac failure can be considered for practical purposes as extracellular fluid which consists of water, sodium, and chloride in constant proportions. So when we speak of a diuretic drug or of diuresis, we imply a substance or process which removes water and sodium chloride and leaves the composition of the remaining fluid in a relatively normal state. The drugs that we use may primarily affect water and, secondarily, salt excretion, or they may primarily affect the excretion of salt and, secondarily, water. Since the composition of body fluid is very closely guarded by the kidney, when one substance is deranged, the kidney usually rapidly readjusts proportions by excreting the related substances.

I plan to discuss diuretic drugs from the physiological point of view under three headings—first, the “acid-producing” salts; second, the xanthines; and, third, the mercurials. Finally I might comment on some of the factors involved in the use of a low salt diet with a large water intake to promote the loss of edema fluid.

### *I. The “Acid-Producing” Salts*

I am going to discuss ammonium chloride as an example because it is commonly used. The history of the acid salts is interesting. They were discovered in this century by German workers who gave calcium

chloride to children to reduce albuminuria, and noticed that they lost weight. Later, about 1920, Haldane used ammonium chloride to improve high altitude tolerance; and in his charts one notices that a marked diuretic effect followed its administration. It was suggested later that calcium chloride and ammonium chloride might be used to remove body fluid. Then the well-known work of Gamble appeared on the effects of calcium chloride and ammonium chloride administration on electrolyte excretion.

Ammonium chloride is usually given by mouth and in enteric coated tablets to prevent gastric irritation. Absorption is rapid and nearly complete in three to six hours, so it is usually given four or five times a day.<sup>1</sup> After absorption from the intestine the  $\text{NH}_4$  ion becomes intimately involved in nitrogen metabolism, disappearing from the blood rapidly. Thus the chloride ion remains and in effect the patient has received hydrochloric acid. The excess chloride ion is excreted by the kidney and thus requires the excretion of base. When the base lost is sodium a proportionate amount of body water is also excreted.

It is instructive to make a brief calculation of the effect of ammonium chloride assuming it were completely efficient; that is, if one molecule of ammonium chloride removed one of sodium. One milliequivalent of ammonium chloride equals .053 grams. Assuming there are 20 liters of extracellular fluid in the average body and 140 milliequivalents of sodium per liter, we have 2800 milliequivalents of sodium in the extracellular fluid of an average person. The question is: How much ammonium chloride would it take to remove all the sodium in a patient's extracellular fluid? The quantity amounts to (.053) (2800) or 148 grams of ammonium chloride: or roughly 10 grams of ammonium chloride a day for two weeks.

Fortunately, the kidney has some defense mechanisms which limit the effectiveness of ammonium chloride and these have an important bearing upon the way in which ammonium chloride should be administered. The base-saving mechanisms of the kidney may largely vitiate the desired action of ammonium chloride. First, the kidney can excrete some of the acid as such, but this mechanism is of limited capacity. More important is ammonium production by the kidney

<sup>1</sup> Often the ineffectiveness of ammonium chloride administration is explained by the appearance of unchanged enteric coated tablets in patient's stools.

which reduces loss of sodium, because the excess chloride is excreted as ammonium chloride instead of sodium chloride. The dynamics of the ammonia-producing mechanism is important. If one gives 8 to 10 grams of ammonium chloride a day to a normal person, on the first day the sodium loss is marked. The second day the loss is not as large and by the third sodium excretion may be nearly normal. If we plot the amount of ammonia produced by the kidney each day, we find that the increase in ammonia production is a sluggish mechanism. Ammonia production rises slowly on the first day and reaches a peak, usually by the third day. And as ammonia production increases there is less sodium loss proportionately. The importance of this is that with normal renal function, initial large doses are more effective than small prolonged doses, since the drug is more efficient in the first few days before ammonia production is fully stimulated.

The drug is more potent and may be much more dangerous with damaged kidneys which do not have a normal ammonia-forming mechanism, because sodium loss can be tremendous. Serious uncompensated derangements of body fluid composition may occur. The kidney may not excrete the chloride. There may be overall depression of function with a low filtration rate and chloride may be retained, producing what may be called a "chloride acidosis". There are changes in the intracellular electrolytes with ammonium chloride administration with a marked loss of phosphorus and potassium, which presumably come from the cells. Also with dehydration and acidosis not only extracellular fluid but intracellular fluid is lost. Thus prolonged administration of "acid producing" diuretic salts is dangerous particularly in a patient with damaged kidneys.

## *II. The Xanthine Diuretics, Caffeine, Theobromine, and Theophyllin*

The xanthines were discovered and first used as diuretics in 1890 by Von Schroeder, and it is worthwhile reviewing some of the things that were discovered by the original investigators. I will omit discussion of caffeine, since it is not usually used as a diuretic. Experimental work on dogs and rabbits with theobromine and theophyllin showed that they were active diuretics, but that their potency varied in different animals. I think it is well to keep in mind in evaluating studies on diuretics, that the cat, rat, rabbit, and human may vary markedly

in their response and that one cannot freely carry experimental conclusions derived from one species to another. Koritschoner and Gram in 1890 pointed out that theobromine showed no toxicity in patients in doses up to 4 to 5 grams a day, and that the theobromine sodium salicylate salt was more soluble than the free compound. They found that theophyllin was a more potent diuretic than theobromine but produced central nervous system, cardiac, and gastro-intestinal disturbances, such as excitement, cardiac irregularities, diarrhea, and nausea and vomiting. Grunwald in 1908 and Butler and Kerpel Fronius in 1935 studied the effect of theobromine on electrolyte balance in rabbits. They found that there was marked loss of sodium chloride and potassium in these animals; the serum became concentrated; there was weight loss; serum non-protein nitrogen rose; and the animals died with a progressive ascending paralysis of the limbs.

There have been two general theories of the mechanism of action of the xanthine diuretics. First that the diuresis is due to an increased renal blood flow; second, that the xanthines cause a decrease in absorption of electrolytes and water in the renal tubule. Without going extensively into the evidence, I think one can be fairly certain that theobromine and theophyllin act on the renal tubule by decreasing reabsorption of sodium chloride. I mention sodium chloride loss first, because the amount of water lost with the xanthines in animals and in patients both with edema and without edema is variable.

One can block the water loss due to xanthines with pitressin but sodium chloride loss in the urine will be as great as with the xanthine alone. The most consistent effect of the xanthines is an increased salt excretion.

Experiments in our laboratory with theophyllin show that the renal blood flow and filtration rate in the dog do not increase. However, an increase in urine flow did occur but it was not the most dramatic effect. The striking effect was the marked increase in the output of sodium chloride due to decreased tubular reabsorption (Fig. 1).

I have selected for comment three clinical evaluations of the xanthines; one by the first group to use xanthines in 1890, the second by Christian in 1916, and the third by Marvin in 1926. Koritschoner and Gram in 1890 studied a series of 38 cases of dropsy, 10 with chronic valvular heart disease, 6 with arteriosclerotic heart disease, 4 with



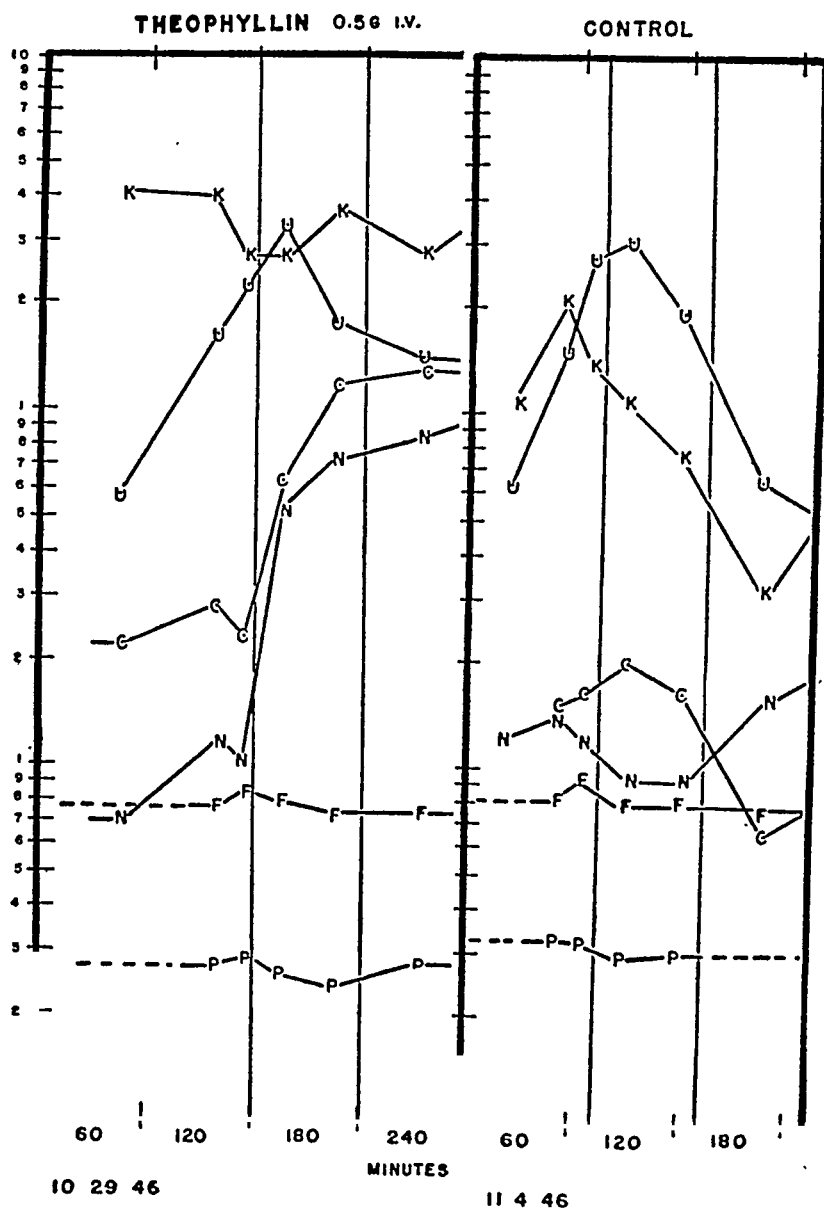


FIG. 1.

P—Renal plasma flow (para-amino hippuric acid clearance)  
 F—Glomerular filtration rate (exogenous creatinine clearance)

degeneration of the myocardium, 3 with cirrhosis of the liver, and 15 with nephritis. They used 4 grams of theobromine a day and concluded that 23 of the 38 patients were markedly benefited, and that the action in the cardiac cases with dropsy was much more effective than in the nephritics. About 1916 Christian conducted a series of experimental and clinical studies on the xanthines. He pointed out that patients who had no edema lost chloride with theophyllin even though there was no diuresis. In five patients with chronic nephritis, 4 of whom had edema, he obtained slight diuresis with 0.3 to 1.0 grams of theophyllin per day, but in all patients there was a marked increase in chloride excretion. He then studied six patients with chronic cardiorenal disease, using theophyllin, 0.3 to 1.5 grams per day, and

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K—Potassium clearance

U—Urine flow

N—Sodium clearance

C—Chloride clearance

The control experiment on the right was made omitting theophyllin to determine any effect of oral creatinine or para-amino hippuric acid infusion. The hour marked by the vertical lines is the comparable time during which theophyllin was infused in the experiment on the left.

The ordinate is a triple cycle logarithmic scale in order that percentage changes in the values are easily compared.

An increase in urine flow occurred in the control experiment probably due to the infusion of para-amino hippuric acid solution, but there were insignificant changes in sodium and chloride clearance during the first two hours.

On the left the specific effect of theophyllin upon sodium and chloride reabsorption in the renal tubule is demonstrated.

During the hour marked by vertical lines 0.5 gram of theophyllin was added to the para-amino hippuric acid infusion, so that the conditions of the experiment were otherwise exactly as on the control day.

During theophyllin infusion, filtration rate (F) and renal plasma flow (P) did not change significantly.

There was a striking increase (nearly ten fold) in the sodium and chloride clearances occurring immediately. Urine flow increased but not significantly more than in the control experiment, and potassium clearance remained approximately the same.

Such a marked increase in sodium and chloride clearance without change in filtration rate is due to decreased reabsorption of sodium and chloride by the renal tubular cells.

Fasting unanesthetized dog on a constant diet. Urine collections made by indwelling catheter.

produced a marked diuresis in four but no diuresis in two who were edema free at the time of administration. There was a marked increase in sodium chloride excretion in those having a diuresis. He concluded at that time that the xanthines were not beneficial in nephritis, but were efficacious in cardiac edema.

In 1926 Marvin studied the effect of diuretics in 77 patients with congestive cardiac failure. He eliminated 36 of the patients who became edema-free on digitalis alone. Of the 41 patients remaining, 23 were benefited with theobromine or theophyllin. He found that theophyllin was more effective than theobromine and that theobromine was sometimes more effective than theobromine sodium salicylate. The patients had untoward reactions, such as nausea and vomiting and some mental excitability with theophyllin, but none with theobromine.

### *III. The Mercurial Diuretics*

The organic mercurials were first used in 1917 by Zeiler and rapidly were heralded as the most powerful diuretics. Mercurial diuretics are usually given by vein or intramuscularly and recently have been given by mouth and rectally. I think most of us use them intravenously or intramuscularly. There has been some recent interesting work on the absorption of the mercurials from muscle. As you know, it is customary to put theophyllin in mercurial preparations. It has been shown by DeGraff that theophyllin mixed with mercurial preparations markedly increases the rate of absorption from the muscle site. Occasionally the effectiveness of mercurials is poor when given intramuscularly, probably because of slow absorption from the muscle in an edematous patient with circulatory failure. Administration by rectal suppository is a convenient and sometimes satisfactory method; but not as uniformly effective as administration intravenously or intramuscularly.

There have been three main theories regarding the mechanism of action of mercurial diuretics. One theory that we can dismiss, I think, is that the mercurials primarily cause mobilization of salt and water from the tissues. The other theories place the site of action in the kidney. Renal blood flow may be increased or there may be decreased reabsorption of salt and water from the renal tubule. The work of Govaerts placed the site of action in the kidney. He showed that if

mercurials were injected into one kidney which was then transplanted to the neck of another animal, the diuresis continued. With the clearance techniques it is found that the mercurials do not primarily affect renal blood flow or filtration rate, but cause a specific decrease of tubular reabsorption of salt and water.

The experimental work of Blumgart and others demonstrates the overall effect of intravenous mercurial injections in normal and edematous cardiac patients. There is a markedly increased excretion of sodium and chloride. There is no change in phosphate, sulfate, or ammonia excretion or in nitrogen balance. There is a loss of extracellular fluid, a diminution in plasma volume and a loss of weight. The serum shows a decrease in chloride which is excreted in greater quantities than in the equivalent amount of body fluid lost.

Doubling the dose of mercurial approximately tripled the amount of fluid lost and doubled the duration of action. Comparison was made with metaphyllin injections (theophyllin-ethylene-diamine or aminophyllin) and with theobromine orally which caused a similar but much less sustained effect.

The effectiveness of the mercurials is influenced by ammonium chloride administration. The augmentation of diuresis obtained by giving ammonium chloride with a mercurial, is more than one would expect from the additive effects of the two when used separately. Also, one can depress the diuresis expected from a mercurial by giving beforehand sodium bicarbonate and producing an alkaline urine. A reasonable explanation of these augmentation and suppression phenomena is that in an acid urine the dissociation of mercury is greater and therefore more readily absorbed by the renal tubule. Ascorbic acid has recently been used in large quantities to augment mercurial diuresis and I suspect that any acid in large quantities may do the same.

The relative usefulness of the mercurials and the xanthines has been frequently discussed. The xanthines, both theobromine and theophyllin, are not as effective as the mercurials. However, the mercurials must be given parenterally for maximal effect whereas the xanthines can be given orally. A few deaths have been reported from the mercurials and there have been rashes, nausea and vomiting observed. Renal damage also may occur after their use. Ordinarily one can

give the xanthines in large doses for several days, although theophyllin in effective doses is likely to have undesirable side reactions. Renal damage from xanthines is not likely.

*Dr. Harvey:* Before we go on to the discussion, Dr. Andrus is going to talk briefly about some of the other techniques that are used in treating the patients with myocardial insufficiency.

*Dr. Andrus:* At the last session we paid particular attention to the early, perhaps primarily responsible, element in the production of congestive heart failure, which we described as myocardial inefficiency. We rehearsed the ability of digitalis to improve the work capacity and, by implication, the function of the myocardium. We mentioned at the time that physiological studies on man had not exonerated the myocardium from its share of responsibility in congestive failure, but had attracted attention to other abnormalities, particularly those of renal function. Dr. Newman has described the armamentarium which is available for the attack on that problem. I hope that he will, in discussion, speak more about the mechanism of the deficient renal function, particularly toward salt, insofar as it is understood, and the influence of diuretics on that.

The condition which is presented by the typical case of congestive heart failure, or "cardiac dropsy", is that of congestion, with a plethora of fluid, and a rather obstinate tendency to retain salt rather than to excrete it normally. I should simply like to mention certain factors which are regarded as important in the clinical management of cardiac failure in the hope of stimulating discussion.

In the first place, although it may be rather impertinent to stress it in this company, there is no therapeutic measure or drug available for the treatment of congestive failure which is a decent substitute for a thorough examination of the patient. The estimation at the beginning, and periodically, of the physical status of the individual is, of course, a primary essential. It is helpful and important to know from the patient's history two important points at least, the story of the progressive development of his disability and, very helpful, what he, himself, has found makes him most comfortable. These small details might seem unimportant but in planning the management of the case they may be very significant.

The first procedure should be rest. There has been a tendency

recently to deprecate the value of rest in the treatment of heart failure. It can be overdone but it cannot be discarded. It should be accomplished in the proper position, not with the patient flat and accumulating fluid in his lungs, but with his body and head raised or with his feet lowered so that if fluid accumulates it may accumulate in his extremities.

These patients are restless and often in great distress. Rest may be difficult to provide without most careful attention to details. A patient can exert himself more, and sometimes more harmfully, struggling with a bed pan than if he gets up to a handy commode. The individual should be assured a normal amount of sleep. Insofar as possible, emotional strains and worries should be relieved. Sedatives will be required and, in the early stages, the opiates are the most effective. In my experience, pantopon is somewhat less liable to produce nausea in these patients than morphine.

With regard to diet, in advanced congestive failure the hazard of the patient taking too much food is not likely to interfere. It is still useful to employ the Karell regime, which prescribes 800 cc of milk. That can be continued for a few days. The diet must be low in salt, because the deficient excretion of salt simply compounds the difficulty of relieving the dropsy. In recent years there has been a return of an early interest in very low salt diets—one to one and a half grams daily—with relatively unrestricted fluids. If such a diet can be made tolerable to the patient it is possible to avoid development of edema, to relieve symptoms considerably.

There are other obvious procedures, which would not escape attention but which are important to emphasize. If fluid has collected in the serous cavities, particularly in the pleural cavities, to remove that by thoracentesis is to confer additional breathing space almost cc for cc.

Additional measures are directed in the advanced cases to the reduction of auricular pressure. Raising the head and lowering the feet will foster a reduction of pressure in the right auricle. Constriction of the extremities by tourniquet is a temporary expedient, useful in acute failure, but it cannot be maintained. Venesection has a more prolonged effect.

The administration of oxygen must be tempered by consideration

of its purpose and its probable effectiveness. It is certainly an effective remedy. When necessary in acute failure it should be administered by mask. There is a good deal of evidence that a mask in which pressure or resistance can be opposed to expiration is more effective than a simple mask carrying on at atmospheric pressure. If oxygen must be administered over a period of days a mask becomes intolerable. Pure oxygen, unless somehow hydrated, becomes irritant. For protracted treatment an oxygen tent is the method of choice.

The therapy with digitalis I shall omit, since that was taken up last week, and Dr. Newman has discussed the use of diuretics. I shall only emphasize that they are more effective in the digitalized patient than in the undigitalized, and that the action of one superimposed upon that of the other may bring much more rapid and much more considerable relief.

Apropos Dr. Newman's historical reference to mercurials, I can't resist mentioning a preparation which is not used now, but which is older than the medical experience of most of us in this room—the Addison-Niemeyer pill which contained digitalis, squills and calomel.

I would like to express an additional word of caution at this point which refers back to my original statement that successive determinations of the physical status of the individual are essential. You can't start a routine on a patient in the state of congestive failure and go away and leave him. Dr. Newman has emphasized that you can't give ammonium chloride indefinitely. This is an outstanding example.

If treatment of the acute stages of congestive failure is effective, the problem then turns to maintenance therapy. This is, in fact, the planning, and testing, and management of a way of life for the patient calculated to maintain his circulatory efficiency at its highest level. Often it is possible for a person to carry on a useful and comfortable existence, even though he has had congestive heart failure. This requires maintenance quantities of digitalis leaf: at least one-tenth, at most two-tenths, gram; in terms of digitoxin two or three, rarely more, tenths of a milligram. It requires habits of rest calculated to diminish the constancy of the strain of existence. Exertion, particularly sudden or violent exertion, must be avoided if the hazards of increased circulatory load are to be escaped. The earliest guiding symptom is in most instances shortness of breath. The outstanding exception is the pa-

tient with aortic stenosis, whose symptoms of overdoing are more frequently those of forward insufficiency of the circulation, fatigue of the legs, general fatigue, fainting after exertion, or cardiac pain. After the patient has recovered, or after the therapeutic effect has been obtained, he often needs to be encouraged to undertake a comfortable degree of exertion. There are, of course, the unhappy cases in which it is not possible, by methods at our disposal, to restore the circulation to permit any useful activity. It is to be hoped that the future may hold a better understanding of circulatory deficiencies which will allow us to do for such patients more than we can do at this moment.

*Dr. Harvey:* The meeting is now open for questions and discussion.

This is a very important subject, one with which everyone very soon begins to accumulate experience. As has been pointed out, there are no strict rules of thumb in handling patients with congestive heart failure and one accumulates skill by long experience; I think therefore that it might be interesting to ask some of the members of the staff here who have had considerable experience in this field to make comments and point out certain important features that have been brought out by the speakers.

*Dr. Bordley:* I would like to ask Dr. Newman to elaborate more on how salt restriction fits into diuretic therapy. It seems to me one of the most important methods of therapy.

*Dr. Newman:* In Marvin's series of cardiac patients, digitalis and salt restriction were sufficient treatment in 36 patients. Probably in the majority of cardiac patients in failure, diuretic drugs are not necessary to free the patient of edema. However edema can be eliminated more rapidly if diuretic drugs are used.

There is a defect in the "cardiac" kidney such that salt is not normally excreted. In a normal person, ingested excess of salt and water is rapidly excreted. In a normal individual on a restricted salt intake, the kidney will conserve salt to a marked degree. In the cardiac even with a large excess of salt and water in the body, an abnormally large fraction of ingested salt is retained; conservation of salt by the kidney is excessive. It has been postulated that cardiac failure results in forward failure in so far as the kidney is concerned and that the diminution in glomerular filtration results in a retention of salt and water. I think this is an interesting suggestion, but I don't think we can



account for edema on the basis of reduced glomerular filtration rate alone. The amount of salt appearing in the urine is the difference between filtration rate and tubular reabsorption. If the filtration rate is diminished, one might just as well blame the tubule for continuing to reabsorb so much salt. In chronic nephritis there is diminution in glomerular filtration, but there is frequently a failure to conserve salt due to decreased tubular reabsorption; in such a case we blame the tubule. In nephrosis there may be supernormal filtration, but there is edema due to excessive tubular reabsorption of salt. Thus in other diseases causing edema there is no direct correlation of filtration rate with salt retention. Tubular reabsorption is an equal if not a governing factor. These patients who have edema in spite of salt restriction and digitalis therapy require diuretic drugs. The most effective drugs are those which decrease the tubular reabsorption of salt;—the mercurials and xanthines, theobromine and theophyllin.

Water in large amounts has been used to treat edema. This practice is based on an old principle. By forcing water and causing a large diuresis one finds eventually that the output of water exceeds the intake. This results from the fact that the kidney is not a perfect organ. The kidney cannot excrete pure water. In other words the tubules cannot remove all the salt nor conserve all the salt in the glomerular filtrate. At a very high urine flow there is a small but definite continuous loss of salt. The composition of body fluid is then protected by losing water also. To make such a regime practical, it is necessary to give enormous quantities of water for several days.

There is certainly no sense in restricting fluid intake in edematous cardiac patients, because a low water intake will aid to some extent in the retention of salt. Restricting fluids means the tubule reabsorbs more water; with conservation of more water it is more likely to reabsorb salt. Fluids should be taken *ad lib.* or up to the point of the patient's tolerance. Restriction of salt not water is of primary importance.

*Dr. Marshall:* Is there any defect in the excretion of potassium by the kidney in cardiac failure?

*Dr. Newman:* From what I have observed there is no inability of the kidney to excrete potassium in cardiac failure, provided there is no primary renal damage.

*Dr. Marshall:* Why wouldn't it be good therapy to put potassium into a low salt diet to make it more palatable?

*Dr. Newman:* Actually we have done that. There is a preparation which has been recently marketed, a salt substitute, which is a mixture of potassium chloride, ammonium chloride and a few other salts that don't contain sodium. It doesn't taste exactly like table salt. However it makes the low salt diets more palatable to many patients and is certainly worth trying. Our dietitians have found it is quite satisfactory also for use in cooking some vegetables and meats.

*Dr. Marshall:* I would like to ask about giving mercurial diuretics every day if necessary or every other day on the assumption that the diuretic is all excreted in 24 hours. I was glad Dr. Andrus brought up the point of the Addison-Niemeyer pill because I have always felt that the action of the organic mercurials was by the same mechanism as the action of calomel. The effect is simply the initial stage of mercurial poisoning, which blocks the absorption of sodium chloride.

*Dr. Newman:* I agree that as far as we know we are giving a renal poison in a mild form.

*Dr. Marshall:* Would there be a definite limit as to how frequently it should be given?

*Dr. Newman:* That subject has recently been commented on by Gold. He suggests giving frequent "non-cumulative" doses.

*Dr. Marshall:* How long does it take the mercury from a single injection to be eliminated? What per cent comes out in 24 hours? Is there any such thing as a "non-cumulative" dose?

*Dr. Newman:* Many patients have been given mercurial injections once or twice a week for one or two years without any evidence of renal damage or other toxicity.

The question of how long the drug can be given would seem to depend on the individual patient's reaction. Certainly one should carefully observe the patient with urinalyses and tests of renal function. The problem of how frequently mercurials should be given and whether accumulation is dangerous is difficult to answer. It is our clinical impression that spacing the injections at twice weekly or weekly intervals gives satisfactory results. That accumulation of mercury in the kidney does occur has been demonstrated recently in dogs and patients. The kidney stores a much higher concentration of mercury

than other tissues analyzed and may retain significant amounts for at least a few weeks after discontinuing the administration.

*Dr. Harvey:* What about the time at which diuretics should be started in the patient with severe congestive failure. There seems to be a growing tendency to start diuretic drugs immediately with other treatment such as digitalis, rest and oxygen. I was of the opinion that diuretics given for congestive failure can actually be harmful if given at too early a stage in the treatment. I think Dr. Andrus answered this in part. He mentioned the fact that bed rest in the proper posture, diet, sedation, and digitalis are, after all, the most important things at the beginning. And perhaps the patient will do very well on that. It may be that renal circulation is so poor in the patient who comes in with untreated failure that diuretics aren't going to be effective.

*Dr. Andrus:* The circulatory effects of digitalis and the action of diuretics will be most fully complementary if diuretics are given after digitalization. It is worth remembering, too, that diuretics do not act immediately and their action persists for hours.

*Dr. Newman:* Certainly a patient should not be given mercupurin at 6:00 PM and be kept awake all night voiding.

*Dr. Marshall:* I would like to add a word about the use of diuretics in the patient who has diminished renal function due to nephritis. We frequently wish to use diuretics in these cases and frequently fear to use them because of possible damage to the patient. I wonder, has anyone had experience with this?

*Dr. Harvey:* Can you answer that, Dr. Newman?

*Dr. Newman:* I think that it has been definitely shown that in active nephritis the acid salts and mercurials result, not always, but usually, in a flare-up of the nephritis with more cells, casts and albumin in the urine, and further depression of function. In a patient with healed nephritis or nephrosclerosis I believe these drugs are relatively safe, but one should proceed cautiously and observe the patient closely. In a patient who is likely to have renal irritation, it is wise to give a small first dose of mercurial slowly. A small test dose has been recommended routinely in all patients by some authorities because of the rare occurrence of other serious toxic reactions. Apparently the xanthine diuretics do not cause significant renal damage even in active nephritis.

While we have at our disposal quite effective methods for the treatment of edema due to cardiac failure, there is much room for improvement in our knowledge and application of these methods and for new drugs. There is need for diuretic drugs like the xanthines causing loss of sodium chloride, but having a greater and longer action with less undesirable side effects. New methods for administration of the mercurials and for augmenting their effect may be developed.

The reasons for the successes or the too frequent failures in therapy can be found only by close clinical observation of the individual patient and by the use of physiological studies and principles.

### SELECTED BIBLIOGRAPHY

#### *General Papers*

- BLUMGART, H. L., GILLIGAN, D. R., LEVY, R. C., BROWN, M. G., AND VOLK, M. C.: Action of Diuretic Drugs. I. Action of diuretics on normal persons. *Arch. Int. Med.* **54**, 40-81, 1934.
- CHRISTIAN, H. A., FROTHINGHAM, C., O'HARE, J. P., AND WOODS, A. C.: Studies of nephritis. *Am. J. Med., Sci.*, **150**, 666, 1915.
- GRUNWALD, H. F.: Beitrage zur physiologie und pharmakologie der niere. *Arch. Exp. Path. u. Pharm.*, **60**, 360, 1909.
- PAGE, I. H.: The action of certain diuretics on the function of the kidney as measured by the urea clearance test. *J. Clin. Invest.*, **12**, 737, 1933.
- WOLF, A. V.: The dehydrating effect of continuously administered water. *Am. J. Physiol.*, **143**, 567, 1945.
- MERRILL, A. J.: Edema and decreased renal blood flow in patients with chronic congestive heart failure; evidence of "forward failure" as the primary cause of edema. *J. Clin. Invest.*, **25**, 389, 1946.
- LYONS, R. H., JACOBSON, S. D., AND AVERY, N. L.: The change in plasma volume and body weight in normal subjects after a low salt diet, ammonium chloride mercupurin. *Am. J. Med. Sci.*, **211**, 460, 1946.

#### *Acid-Base Equilibrium*

- BLUMGART, H. L., GILLIGAN, D. R., AND VOLK, M. C.: Effect of diuretic drugs on acid-base equilibrium in patients with cardiac edema. *Medical Papers. Christian Birthday Volume*, 191-203, 1936.
- PALMER, W. W., AND HENDERSON, L. J.: A study of the several factors of acid excretion in nephritis. *Arch. Int. Med.*, **16**, 109, 1915.
- VAN SLYKE, D. D., LINDER, G. C., HILLER, A., LEITER, L., AND MCINTOSH, J. F.: The excretion of ammonia and titratable acid in nephritis. *J. Clin. Invest.*, **2**, 255, 1926.
- GAMBLE, J. L., BLACKFAN, K. D., AND HAMILTON, B.: A study of the diuretic action of acid producing salts. *J. Clin. Invest.*, **1**, 359, 1925.

- BLUM, L., AUBELL, E., AND HAUSKNECHT, R.: L'Action diuretique des sels de calcium dans les oedemes generalises. Bull. et. mem. Soc. Med. d. Hop. de Paris, **45**, 1561, 1921.
- HALDANE, J. B. S.: Experiments on the regulation of the blood's alkalinity. J. Physiol., **55**, 265, 1921.
- HALDANE, J. B. S., HILL, R., AND LUCH, J. M.: Calcium chloride acidosis, J. Physiol., **57**, 301, 1923.

### *Xanthines*

- CHRISTIAN, H. A.: The effect of theobromine sodium salicylate in acute experimental nephritis as measured by the excretion of phenolsulphonphthalein. Arch. Int. Med., **14**, 829, 1914.
- CHRISTIAN, H. A., AND O'HARE, J. P.: A study of the therapeutic value of a diuretic (theobromine sodium salicylate) in acute experimental nephritis. Arch. Int. Med., **11**, 517, 1913.
- CHRISTIAN, H. A.: Some studies of a diuretic (theocin). Arch. Int. Med., **18**, 606, 1916.
- GRAM, C.: Klinische versuche uber die diuretische Wirkung des theobromin. Therapeut. Monatshefte, No. 4, 10, 1880.
- KERPEL-FRONIUS, E., AND BUTLER, A. M.: Salt and water loss in diuretin diuresis and their relation to serum NPN and electrolyte concentration. J. Exp. Med., **61**, 157, 1935.
- KORITSCHONER, M.: Klinische versuche uber dus diuretin "Knoll" (theobromine-natriosalicylicum) Wien. Klin. Wchnschr., **3**, 753, 1890.
- MARVIN, H. M.: The value of xanthine diuretics in congestive heart failure. J. A. M. A., **87**, 2043, 1926.
- VON SCHROEDER, W.: Uber die diuretische wirkung des caffeins und der zu derselben kruppe gehorenden substanzen. Archiv. fur Exp. Path. und Pharm., **24**, 85, 1888.
- VON SCHROEDER, W.: Uber die wirkung des caffeins als diureticum. Arch. fur Exp. Path. und Pharm., **22**, 39, 1886.

### *Mercurials*

- Conference on Therapy. The dose of a drug. Am. J. of Med., **2**, 296, 1947.
- CRAWFORD, J. H. AND MCINTOSH, J. F.: Observations on the use of Novasurol in edema due to heart failure. J. Clin. Invest., **1**, 333, 1925.
- DE GRAFF, A. C., BATTERMANN, R. C., AND LEHMAN, R. A.: Influence of theophylline upon absorption of mercupurin and salyrgan from the site of intramusc. injection. J. Pharm. and Exp. Therap., **62**, 26, 1938.
- FARNSWORTH, E. B.: Clearance of inulin, diodrast, chloride and phosphate under mercurial diuresis. Am. J. of Med., **1**, 246, 1946.
- GOVAERTS, P.: Origine renal on tissulaire de la diurese pur un compose mercuriel organique. Comp. Rend. Soc. Biol., **99**, 647, 1928.

- HARGER, R. N., BENNET, J. R., HULPIEN, H. R., AND SCHNEIDER, H. J. Tissue storage of mercury following repeated administration of organic mercury diuretics or mercuric chloride. *Fed. Proc.* 6, No. 1, part 2, 258, 1947.
- LANG, E. P., KUNZE, F. M., AND FITZHUGH, O. G.: Mercury storage in the rat following ingestion of mercuric acetate and phenyl mercuric acetate. *Fed. Proc.*, 6, No. 1, part 2, 349, 1947.
- LYONS, R. H., AVERY, N. L., AND JACOBSON, S. D.: Effect of dehydration produced by mercupurin on plasma volume of normal persons. *Am. Heart J.*, 28, 247, 1944.
- ZIELER, K.: Novasurol ein neues quecksilbersalz zur syphilisbehandlung mit bemerkungen uber die grundsätze der quecksilberbehandlung. *Munch. Med. Wchnschr.*, 64, 1257, 1917.
- ETHRIDGE, C. B., MYERS, D. B., FULTON, M. N.: The modifying effect of various inorganic salts on the diuretic action of salyrgan. *Arch. Int. Med.*, 57, 714, 1936.

# DIFFUSE CONGENITAL CYSTIC HYPERPLASIA OF STOMACH CLINICALLY SIMULATING CARCINOMA\*

## REPORT OF A CASE

H. WILLIAM SCOTT, JR., AND TORRENCE P. B. PAYNE

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Exclusive of peptic ulcer, benign lesions of the stomach simulating carcinoma are relatively uncommon. It has been estimated that less than 0.5 per cent of gastric tumors are benign (1). Among non-neoplastic lesions, chronic gastritis, syphilis, and tuberculosis occasionally have been known to resemble gastric cancer. We have recently encountered a case of diffuse cyst formation involving the mucosa and submucosa of the stomach which clinically simulated gastric carcinoma.

### *Case Report*

J. M. (408483), a 58 year old colored laborer, was first admitted to the Urological Service of the Johns Hopkins Hospital on January 13, 1947, because of suspected left renal tumor. Family history was irrelevant. Past history showed gonorrhea at age 19 and scarlet fever at 21; otherwise there had been no significant illness and the patient had been well and active until the onset of the present illness.

Two years before admission the patient developed the first of a series of episodes of dull, aching periumbilical pain radiating to the left lower quadrant. These pains had no relation to meals and were of an intermittent character, occurring every two to three months and persisting for three to four days at a time. There was no associated nausea or vomiting. In the six months prior to admission he had poor appetite and lost about 18 pounds in weight. During this time he had occasional epigastric pains from which he obtained some relief by taking bicarbonate of soda. He had become constipated and had been bothered by hemorrhoids for several months. Six weeks before admission he had developed burning on urination in addition to the other complaints. Because of a suggestive filling defect in the upper pole of the left kidney, found on intravenous pyelography in his dispensary studies, he was admitted to the Urological Service. Retrograde pyelograms were then performed and were entirely normal. There was microscopic hematuria and pyuria which was thought to be on the basis of chronic prostatitis. A gastro-intestinal series showed a peculiar filling defect in the antrum of the stomach, and the patient was transferred to the surgical service.

\*From the Departments of Surgery and Pathology of the Johns Hopkins School of Medicine and the Johns Hopkins Hospital.

*Physical Examination.* T. 98.6, P. 96, R. 20, B.P. 118/75. The patient was a thin, emaciated, elderly colored man who was partially edentulous. Nose and throat showed no abnormalities and there was no lymphadenopathy. The lungs were clear and the heart was within normal limits. The abdomen was scaphoid, non-tender, and there were no palpable masses. Rectal examination showed external hemorrhoids and a small firm prostate.

*Laboratory Studies.* Hemoglobin was 12 gms. per cent; W.B.C. 5,200 with normal differential. Urine contained a few WBC and an occasional RBC per high power field but was otherwise negative. P.S.P. showed 76 per cent excretion in 2 hours. N.P.N. was 33 mgm. per cent; Chloride 98 milli-equivalents/liter; CO<sub>2</sub> combining power 30 milli-equivalents/liter. Serological test for syphilis was negative. Gastric analysis showed a total acid of 12 clinical units and no free acid. Stools were guaiac negative. X-ray of the chest revealed moderate emphysema, but was otherwise not remarkable. Cholecystograms and barium enema showed no abnormalities. Gastro-intestinal series showed a large rounded lesion in the anterior wall of the stomach in the antral region (Fig. 1). There was no ulceration of the mucosa over this lesion. There were many small areas of thickening of the gastric wall adjacent and extending some distance from the rounded shadow. Peristalsis was impaired by these changes, but the stomach emptied fairly well within 5 hours. An infiltrating carcinoma of the stomach was felt to be the most likely diagnosis.

*Operation (H.W.S., Jr.):* Under ether anesthesia, the abdomen was entered through an upper abdominal midline incision. There was no free peritoneal fluid. Abdominal exploration revealed no metastases in the liver, which was normal in appearance. There were no gross abnormalities of the large or small bowel. Examination of the stomach showed it to be moderately dilated but the gastric wall was normal in external appearance with no visible evidence of ulcer or tumor. On palpation there was a diffuse rubbery thickening of the gastric wall extending from the pylorus to the mid-portion of the body of the stomach. There was a sharp line of demarcation at the pylorus, and the duodenum was normal in appearance and quite mobile. The thickened gastric wall was not indurated, and there was a fairly sharp upper border at about the mid-portion of the body of the stomach above which the texture of the gastric wall seemed normal. A biopsy was taken of the full thickness of the wall of the stomach in the antral region. Frozen section showed no evidence of carcinoma. On extending the biopsy opening, the stomach wall was found to be filled with small cysts ranging up to the size of a pea and filled with watery mucoid fluid. The mucosa was not inflamed or ulcerated, but the gastric lumen in the antral region was not much larger in caliber than the operator's index finger.

Since the thickened mucosa tended to obstruct the emptying of the stomach and since we could not be positive that we were dealing with a benign lesion, we decided to do a partial gastrectomy. The greater and lesser curvatures of the stomach were then mobilized and the distal two thirds of the stomach were re-



sected. It appeared grossly that we were transecting the stomach at least 3 inches proximal to the upper border of the hypertrophied gastric wall. An anticolonic gastrojejunostomy of the Polya type was then constructed and the abdomen closed.



FIG. 1. GASTRO-INTESTINAL SERIES. NOTE FILLING DEFECT IN ANTRAL REGION

*Course:* After operation the patient was maintained on gastric suction for 24 hours and parenteral fluids were given for 3 days. Graduated oral feedings were taken well and convalescence was entirely uneventful. Postoperative gastro-intestinal series showed a satisfactory stoma with no retention. The patient was discharged from the hospital on the 16th postoperative day. In the 5 months since discharge he has been followed in the G.I. clinic and has been maintained on

a full soft diet with intermediate feedings. He has gained 12 pounds in weight, has remained entirely asymptomatic, and is back at his accustomed work.

*Pathological Study (T.P.B.P.)*

*Gross Description:* The specimen (S.P. 47-218) consists of the distal 15 cms. of the stomach. The serosa is smooth and pink. Along the greater curvature in the prepyloric region there is a fresh linear incision measuring about 5 cms. in



FIG. 2. Distal end of resected stomach. Diffuse cystic change in submucosa. Small abscess in submucosa with surrounding scarring. Continuity between epithelium of cysts and surface epithelium of mucosa (A).

length and having six black silk sutures in it; in this region the wall of the stomach feels thicker than elsewhere. By extending this incision the stomach is opened along its greater curvature. The mucosa does not have the usual rugose appearance but is lifted up into somewhat thicker folds; one of these folds along the greater curvature in the pyloric region is especially prominent. No ulcers or erosions of the mucosa are seen. The gastric wall is thicker than usual, and in the

area of the previously described incision measures about 1 cm. in thickness. In this area several cystic spaces, measuring about 5 mm. or less in greatest diameter, are seen lying beneath the mucosa. On careful examination small cystic spaces are seen scattered throughout the entire wall of the stomach. Representative blocks are taken for microscopic study.

*Microscopic Description:* In all of the sections there are cystic spaces of varying size lined by columnar or cuboidal epithelium and situated in the mucosa or sub-



FIG. 3. HIGHER POWER OF (A) FIGURE 2. NOTE GLANDULAR OUTPOUCHINGS AROUND CYSTS

mucosa. In some areas definite continuity can be traced between the epithelium of the cysts and the surface epithelium of the mucosa (Figs. 2 and 3). In several of the larger cysts there is intracystic papillary formation (Fig. 4). Small glandular outpouchings, lying in a myxomatous stroma, arise from others (Fig. 2 and 3). Many of these larger cysts have a delicate layer of smooth muscle around them, as if they had not completely penetrated the muscularis mucosae, while others are quite definitely in the submucosa. In all of the sections this diffuse cystic change

is seen and it is clear that the process must have extended beyond the limits of the resected stomach. There is no inflammation or scarring (Fig. 5) except in the sections taken from the thickened area in the prepyloric region of the stomach. Here in the submucosa a small abscess is seen (Fig. 2). It is apparently formed within one of the larger cystic cavities, for fragments of intact epithelium are still present around the periphery where, in addition, there is early fibroblastic prolifera-



FIG. 4. DISTAL END OF RESECTED STOMACH. CYSTS WITH PAPILLARY FORMATION. EDGE OF ABSCESS ON RIGHT

tion. Among the numerous polymorphonuclear leucocytes, bacterial stains reveal no definite organisms, but several gram positive fragments are seen which may represent the relics of digested bacteria. Collections of acute inflammatory cells are also seen in several adjoining cystic spaces, and the surrounding stroma is infiltrated with them (Figs. 2, 3 and 4). In addition, there is definite scarring of the submucosa in this region. In none of the sections is any erosion of the mucosal epithelium seen.

*Final Diagnosis:* Congenital cystic hyperplasia with abscess formation in stomach.

*Comment:* While the most interesting features of this case are the cystic changes in the mucosa and submucosa of the stomach, there was a striking similarity between the clinical aspects and those of gastric carcinoma. In retrospect, it is difficult to establish any signi-

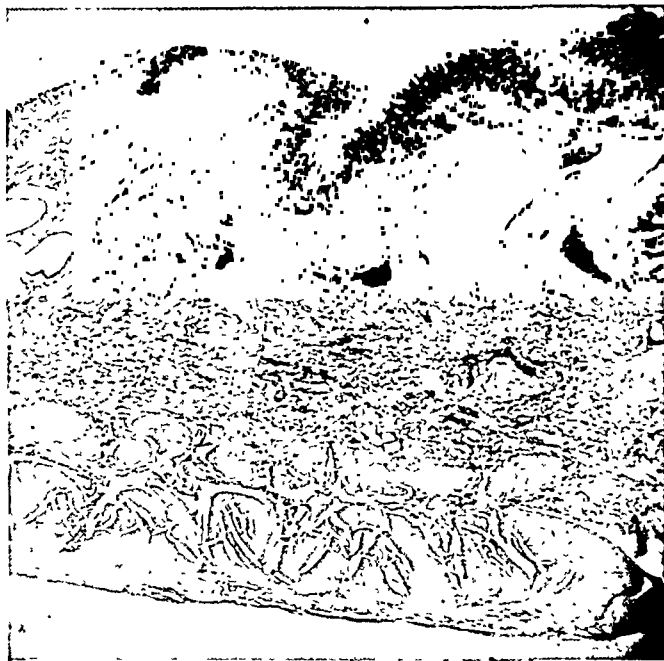


FIG. 5. PROXIMAL END OF RESECTED STOMACH. DIFFUSE CYSTIC CHANGE IN SUBMUCOSA. THERE IS NO INFLAMMATION OR SCARRING HERE

ficant points of differentiation. In an elderly man with anorexia, weight loss, epigastric pain, gastric anacidity, and a filling defect in the gastric antrum on roentgen examination, a diagnosis of carcinoma should be made. It is unfortunate that preoperative gastroscopy was not performed in this patient, but, regardless of what it might have disclosed, exploration would doubtless have been necessary. Even at operation there was considerable doubt as to the benign nature of the lesion.

Pathologically there was no evidence of any neoplastic process and the diffuse cystic change in the mucosa and submucosa was, as far as we are aware, unlike any previously described lesion (2). In chronic follicular gastritis there may be epithelial hyperplasia with cyst formation in the gastric mucosa (3). Cystic changes are also occasionally seen in the edges of chronic peptic ulcers. In this case, however, there was no evidence of any previous inflammatory process or ulceration. The cystic change was diffuse with definite papillary formation and probably involved the whole stomach. It was only in the antrum that there was any evidence of inflammation and this was relatively recent, well localized, and undoubtedly represented infection occurring secondarily in one or several of the cysts. This probably resulted in the more recent symptoms.

This condition appears to represent a developmental defect in the formation of the gastric glands which was present since birth. As far as is known there was no associated cystic disease in lungs, liver, pancreas or kidneys.

#### BIBLIOGRAPHY

- (1) BOYD, W.: Surgical Pathology; W. B. Saunders Company, Philadelphia, 1939.
- (2) HENKE, F. UND LUBARSCH, O.: Handbuch der Speziellen Pathologischen Anatomie und Histologie; Julius Springer, Berlin, 1933.
- (3) FITZGERALD, R. R.: Chronic Follicular Gastritis; British Journal of Surgery, 19: 25, 1931.

## BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers  
and not necessarily those of the members of the Editorial  
Board of this Journal)

*An Integrated Practice of Medicine.* By HAROLD THOMAS HYMAN. Illus. 4131 pp. \$50.00 W. B. Saunders Company, Philadelphia, Pennsylvania, 1946.

This "Integrated Practice of Medicine" was written by a group of practitioners expressly for the guidance of the general practitioner. It is an extensive work consisting of four volumes and a separate comprehensive index. The ambitious aim of the senior author was to place in the hands of the general practitioner a single authoritative reference source devoted not only to the subject matter of internal medicine but of all the associated fields of general medicine, and a survey of the preclinical sciences in addition. Among others, this broad scope includes such diversified topics as psychiatry, dentistry, obstetrics, emergency major surgery, rehabilitation, immunology, chemistry, radiology, dietetics, and therapeutics. The plan of the author was to integrate the data of this complex material as they pertain to the specific complaints presented by the patient to the general practitioner in order that he might become "the complete physician". To facilitate the correct interpretation of symptoms and signs, 318 tables of differential diagnosis have been prepared.

The magnitude of such an undertaking is manifest, and one marvels at the authors' intrepidity. To fulfill its purpose, such a work would need to be comprehensive yet adequately detailed, entirely up to date, and thoroughly sound in its opinions. Although possessing these attributes to a considerable degree, this work unfortunately possesses none of them to a sufficient degree. The authors have endeavored to encompass too much in too small a space, employing a format which does not permit additions to the text as new developments appear. Thus Loeffler's syndrome is disposed of in four lines as an acute pneumonitis occurring frequently in patients with vasomotor rhinitis and bronchial asthma. Only four lines are devoted to folic acid, no mention being made of its use in the treatment of sprue. The newer anti-malarial agents are not discussed. In speaking of the toxicity of streptomycin, reference is not made to the vestibular reactions which may occur, and the statement is made that "toxicity is negligible and bacterial fastness unusual". In discussing the treatment of acute rheumatic fever, it is related that "vitamin P obtained from red pepper and lemon peel is said to control the disease", and "there is abundant evidence to suggest that the salicylates inhibit the antigen-antibody reaction that is responsible for the vascular and connective tissue injury in acute rheumatic fever". But four lines are devoted to the dietary treatment of disturbances of the circulation, there being no discussion of the advantages of salt restriction. Many would disagree that "only a small pro-

portion of the patients with congestive heart failure with sinus rhythm is favorably influenced" by digitalis. In considering the treatment of subacute bacterial endocarditis with penicillin, the use of heparin is advised without reservation, it being said that "... nor have there been untoward consequences as the result of the increase in bleeding time". Not all would agree that "true lobar pneumonia a relatively uncommon disease" or that "the present drug of choice is sulfadiazine" in its treatment. A single line is devoted to rheumatic pneumonia in which the impression is given that the entity occurs only "in a small number of the most severely ill patients". More recent experiences with anti-biotics already requires rewriting of the sections devoted to the treatment of lung abscess and empyema, which are described as being amenable only to surgery. In a table of "Confirmatory Diagnostic Aids in Respiratory Infections", a sputum culture is advised when tularemia or psittacosis is suspected, and the Weil Felix reaction is recommended for Q fever. The cold agglutinins test is not listed for virus pneumonia.

It is the opinion of the reviewer that the general practitioner would find a more abundant and stimulating source of information and help in the classical texts devoted to medicine, pharmacology, physiology, and clinical biochemistry than is contained in these four volumes.

P. A. T.

*Anesthesia in General Practice.* By STUART C. CULLEN. Illus. 260 pp. \$3.50. The Yearbook Publishers. Chicago, Illinois, 1946.

This short volume is a fitting companion to the handbook series. As is proper, the emphasis is upon the practical aspects of anaesthetic practice and not on theoretical considerations. In every sense it is a manual directed to medical students and those others of the profession whose interest in anaesthesia is general. The text is presented in a simple, straightforward manner. In addition to the usual discussions of agents and technique, oxygen therapy and pre- and post-anaesthetic management are discussed. There is a short but adequate discussion of curare by the author who is also one of the outstanding authorities on the clinical application of this drug in anaesthesia.

The book is characterized by a most sensible attitude toward the subject. The material is up-to-date. An otherwise soporific subject is relieved by some lively cartoons.

M. H. H.

*The Diagnosis and Treatment of Bronchial Asthma.* By LESLIE N. GAY. Illus. 334 pp. \$5.00. Williams & Wilkins Company, Baltimore, Maryland, 1946.

A book must not be read or criticized before the preface is carefully scanned. The author makes it very clear that except for the chapter on Physiology of Respiration the contents of the remaining chapters represent a written record of his experiences, impressions, opinions and advice. For those of us who have been closely associated with Dr. Gay, the reading of this book produces all the pleasant sensations that enshroud nostalgia.



It is quite true that the treatment of pollens as causes of asthma smacks of provincialism and therefore the internist and allergist west of the Mississippi River will be left hungry for information. Nevertheless, the treatise was not intended to be the usual type of textbook, and such a criticism is unjust. The same reasoning may be withheld in view of the author's "narrow-mindedness" in regard to food hypersensitivity. The brevity of his discussions on food allergy and its place in the etiology of asthma parallels exactly his personal attitude in regard to the smallness of its importance.

The chapter on the Pathology of Asthma is tedious to read, with all the case reports and autopsy findings. But still, the information contained in the many reports represents a world of information to be appreciated to its fullest by "students". This section of the book will long outlive the author.

The section on treatment is complete in every respect. Though the discussions on helium might well have been abridged, still the recording of interesting information is always pleasing, and the picture of the development of helium that is written gives one a solid footing for its appreciation. If the reader cares nothing for background, however, he will become impatient with the author and with his resourcefulness and thoroughness for detail.

The book is everything that it is intended to be, and for those who respect the author and wish to have his counsel, they will find in his work complete satisfaction.

E. L. K.

*Essentials of Endocrinology.* By ARTHUR GROLLMAN. 2nd Edition. Illus. 644 pp. \$10.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.

In 1941 the first edition of Grollman's *Essentials of Endocrinology* was published. Concepts concerning many endocrine disorders had been based upon fanciful and ill-founded conjectures rather than experimentally established, scientific facts. The author attempted to approach the clinical aspect of the subject from a sound scientific background. To do this, it was necessary to review and critically evaluate a vast amount of literature and to correlate this into an orderly exposition of the anatomy and functions of the endocrine glands. On the whole the results were splendid and the conclusions sound.

In the past six years there has continued to be a great deal of experimental investigation in endocrine physiology, although the basic concepts have not been materially altered. Of even greater importance, there has been an increased application of newer methods of study and hormone assay to the problems of clinical diagnosis. In the second edition the author has introduced a considerable amount of the new material. Of note are a chapter of 23 pages on "The Hypothalamus and Its Disorders" and discussions of exophthalmic ophthalmoplegia, thiouracil therapy and alloxan diabetes.

A number of Grollman's conclusions are still open to question. He states that the hypothalamus influences gonadal function not by direct action of the hypophysis but rather by nervous impulses to the gonad itself. Likewise, it is stated

that the available evidence indicates that a single compound is responsible for all of the activities of the normal adrenal cortex and that only in pathologic disorders is androgen produced. The various corticosterones and androgens which have been isolated from the adrenal or from the urine of castrates are believed to be artefacts or end metabolites. In the section on sex differentiation ambisexual potentials and genetic mosaics are well discussed, but the reader is confused by the statement on p. 449 that "in the human, intersexes are always genetically female" and on p. 451 that the sex aberration which occurs most frequently is male pseudohermaphroditism in which the abnormal individual is genetically a male.

For the student of endocrinology the writer has done an excellent service in presenting in one volume a well-organized and clearly written compilation and analysis of basic facts, documented with fairly abundant references. From the standpoint of the clinician one regrets that the author does not make a greater effort to point out the application of the newer methods of study to the differential diagnosis of the etiology of various symptom complexes such as sexual precocity and sexual infantilism. For instance, as carried out in most laboratories, the follicle-stimulating-hormone assay has served to differentiate accurately pituitary infantilism from primary hypogonadism. The writer states that the test is of little practical value. It is true that the F.S.H. assay as ordinarily performed does not distinguish between different types of gonadotrophin and that the 17-ketosteroid excretion does not measure precisely androgenic activity. Nevertheless these tests are important tools in the armamentarium of the endocrinologist and have been of aid in clinical diagnosis. The writer does not emphasize sufficiently the fact that many symptom complexes may be caused by a lesion either of the hypothalamus, in the pituitary or in one of the "target glands". There is need for a text which will guide the clinician in the methods of study to determine the site of the lesion. In a book which is otherwise so complete one is surprised that there is no adequate description of some of the clearcut but recently described disorders such as the syndrome of ovarian agenesis and dwarfism, and that associated with the primary hyaline degeneration of seminiferous tubules. One misses a discussion of the general metabolic effects of the androgens. Although he is correct in divorcing clinical diagnosis from the fanciful concepts of the past, it is to be regretted that so able a writer as Grollman does not make clearer the application of modern methods of study in the differential diagnosis of endocrine disorders.

L. W.

*Gynecology Including Female Urology.* By LAWRENCE R. WHARTON. 2nd Ed. Illus. 1027 pp. \$10.00. W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.

As stated in the preface, the second edition of Wharton's *Gynecology Including Female Urology* incorporates a number of revisions and additions which serve to increase the usefulness of this already valuable text and bring it up to date along those lines in which the most recent advances have been made.

In that portion of the book devoted to gynecology, notable changes are to be

found in the two chapters on embryology and congenital malformations. These have been rewritten and enlarged to include such subjects as sex differentiation in early embryonic life and the recent studies on sexual infantilism resulting from agenesis of follicular elements in the ovary. Likewise, the chapter dealing with disorders of ovulation and ovarian function now includes a discussion of the significance and clinical importance of changes in the basal temperature curve associated with ovulation.

The phenomenon of precocious menstruation, its causes and treatment, is dealt with at some length, and is a new and interesting addition to the chapter devoted to the general subject of menstruation.

The section on female urology has been improved by some rearrangement of the subject matter and the addition of two new chapters. One of these describes the technique of water cystoscopy, the various uses of different types of water cystoscopes, and the application of this technique as a valuable adjunct or alternate to the Kelly air method in the study and treatment of urinary conditions in women. The other new chapter, devoted to the female urethra, properly emphasizes the clinical importance of the anatomy, structure, and relationships of this unit in considering the various abnormalities, diseases, and injuries to which it is subject.

In dealing with the problem of the surgical removal of ureteral stones, the author has amplified his original description of the technique of ureterotomy in the upper middle, or lower thirds of the ureter, and supplemented the text with excellent illustrations.

At the end of the book, two important chapters have been added, one dealing with chemotherapy and the other with irradiation and its application to gynecology and female urology. The first of these is a clear and concise resumé of the present state of our knowledge concerning the actions, uses, and toxic manifestations of the sulfonamides and mandelic acid, followed by an equally good discussion of penicillin and other antibiotic agents. The second combines very logically the generally accepted views concerning the proper use of x-ray and radium in dealing with various gynecological conditions and with tumors of the urinary tract. Controversial points are brought out and complications following irradiation are freely discussed.

These and other revisions serve to retain the position of this book as a leading authoritative text which may be read with profit by student and practitioner alike.

E. H. R., Jr.

*Techniques and Procedures of Anesthesia.* By JOHN ADRIANI. Illus. 404 pp. \$6.00. Charles C. Thomas, Springfield, Ill. 1947.

In a technique reminiscent of his popular "Pharmacology of Anesthesia," Dr. Adriani has again employed the outline form to good advantage. Though not so profusely illustrated as the former volume, it is not quite so sparing of words in the actual text. Comments, reasons, advantages and disadvantages of the subjects under discussion are set down in an orderly 1, 2, 3, fashion. In this way the author achieves a remarkable clarity of text and eliminates the dross of words

that make technical texts difficult. The coverage of the material is complete, all forms of anaesthesia are dealt with, inhalational, regional, rectal intravascular. In addition resuscitation and oxygen therapy are briefly surveyed. Again in this volume, Dr. Adriani draws attention to the mechanical equipment, machines, tanks, valves, etc., a subject too often neglected.

This book will be useful to those actively engaged in either learning or teaching anaesthesia. For the former, its clarity will introduce some order into steps of technique and for the latter it will provide an organized outline useful for teaching. It is however, "cook-book" anaesthesia and will only be a way station for those seriously interested in the subject. The illustrations and tables are satisfactory. The main virtue of this book lies in the severe clarity and orderliness of the material presented.

M. H. H.

## BOOKS RECEIVED FOR REVIEW

- Advances in Internal Medicine.* Vol. 2. Editors: WILLIAM DOCK and I. SNAPPER. Illus. 642 pp. \$9.50. *Interscience Publishers, Inc., New York, New York, 1947.*
- Advances in Pediatrics.* Vol. 2. Editors: S. Z. LEVINE, ALLAN M. BUTLER, L. EMMETT HOLT, JR., and A. ASHLEY WEECH. Illus. 407 pp. \$6.75. *Interscience Publishers, Inc., New York, New York, 1947.*
- American Illustrated Medical Dictionary, The.* By W. A. NEWMAN DORLAND. 21st ed. Illus. 1660 pp. \$8.50. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.*
- American Sanatorium Association, a Brief Historical Sketch.* By LEWIS J. MOORMAN. Illus. 72 pp. \$1.00. *National Tuberculosis Association, New York, 1947.*
- Atlas of Cardiovascular Diseases.* By IRVING J. TREIGER. Illus. 180 pp. \$10.00. *The C. V. Mosby Company, St. Louis, Missouri, 1947.*
- Bone and Bones: Fundamentals of Bone Biology.* By JOSEPH P. WEINMANN and HARRY SICHER. Illus. 464 pp. \$6.00. *The C. V. Mosby Company, St. Louis, Missouri, 1947.*
- Cambridge Medical History.* By SIR WALTER LANGDON-BROWN. 119 pp. *Cambridge University Press, New York, New York, 1947.*
- Clinical Radiology. A Correlation of Clinical and Roentgenological Findings.* Edited by GEORGE UTLEY PILLMORE. 2 vols. 1600 pp. Illus. \$45.00. *F. A. Davis Company, Philadelphia, Pennsylvania, 1946.*
- History of the American Medical Association, 1847-1947.* By MORRIS FISHBEIN. Illus. 1226 pp. \$10.00. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.*
- Infant Feeding with Evaporated Milk.* Illus. 40 pp. *Evaporated Milk Association, Chicago, Illinois, 1947.*
- Infant Nutrition: A Textbook of Infant Feeding.* By P. C. JEANS and WILLIAMS MCKIM MARRIOTT. 4th ed. 516 pp. \$6.50. *C. V. Mosby Company, St. Louis, Missouri.*
- Insides Out.* By JOHN MASON BROWN. Illus. 202 pp. \$2.00. *Whittlesey House, McGraw-Hill Book Company, Inc., New York, New York, 1947.*
- Internal Medicine in General Practice.* By ROBERT PRATT MCCOMBS. 2nd ed. Illus. 741 pp. \$8.00. *W. B. Saunders Company, Philadelphia, Pennsylvania, 1947.*
- Medical Writings of Anonymus Londinensis.* By W. H. S. JONES. 168 pp. \$2.75. *Cambridge, at the University Press; The Macmillan Company, New York, New York, 1947.*
- Methods of Diagnosis.* By LOGAN CLENDENING and EDWARD H. HASHINGER. Illus. 868 pp. \$12.50. *The C. V. Mosby Company, St. Louis, Missouri, 1947.*

- New and Nonofficial Remedies: Issued Under the Direction and Supervision of the Council on Pharmacy and Chemistry of the American Medical Association.* 749 pp. \$3.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.
- Nursing.* By LULU K. WOLF. Illus. 534 pp. \$3.50. D. Appleton-Century Company, New York, New York, 1947.
- Osteotomy of the Long Bones.* By HENRY MILCH. Illus. 294 pp. \$6.75. Charles C. Thomas, Springfield, Illinois, 1947.
- Practical Emulsions.* By H. BENNETT. Illus. 568 pp. \$8.50. Chemical Publishing Company, Brooklyn, New York, 1947.
- Preoperative and Postoperative Care.* By WILLIAM J. TOURISH and FREDERICK B. WAGNER, JR. Illus. 338 pp. \$6.00. F. A. Davis Company, Philadelphia, Pennsylvania, 1947.
- Principles and Practice of Medicine, The.* By HENRY A. CHRISTIAN. 16th ed. 1539 pp. \$10.00. D. Appleton-Century Company, Inc., New York, New York, 1947.
- Signs and Symptoms.* By CYRIL M. MACBRYDE. Illus. 439 pp. \$12.00. J. B. Lippincott Company, Philadelphia, Pennsylvania, 1947.
- Textbook of Physiology.* By WILLIAM D. ZOETHOUT and W. W. TUTTLE. 9th ed. Illus. 723 pp. \$4.75. The C. V. Mosby Company, St. Louis, Missouri, 1946.



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